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# NOTE ON AN INDIRECT EFFECT OF SPRAYING POTATOES WITH BORDEAUX MIXTURE

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Application of copper sprays to Irish potatoes has long been advocated for the control of the early and late blights, as well as for a supposed physiological effect of copper on the plants, whereby they are kept in a green, growing condition for a longer time than unsprayed plants. More recently attention has been called to the use of Bordeaux mixture for the control of tip-burn, by Erwin (3), and for the prevention of hopper-burn, by Dudley and Wilson (1). However, a spraying experiment on the horticultural grounds at Columbia, Missouri, conducted during the spring and summer of 1921, indicated that, under certain conditions, the application of Bordeaux or other spray having similar physiological effects may produce indirect results which are undesirable.

The variety used was Early Ohio, a good strain of which was obtained from northern Michigan and planted March 17, under uniform conditions. Four applications of 4-4-50 Bordeaux were made during May and June, both with and without arsenate of lead and nicotine sulphate. The sprayed plants remained green about three weeks longer (see fig. 1), and yielded an average of 34.2 percent more, than the checks, when dug August 28th. Since neither early nor late blight was present, but leafhoppers were numerous as usual after July 1, these results may be ascribed to tip-burn and hopper-burn control, and perhaps to other physiological effects. However, the tubers from the sprayed plots consisted to a large extent of knobby second growths, so that the actual quantity of marketable potatoes was really much less than from the check plots, the tubers of which showed second growth only to a moderate extent (see fig. 2).

The Early Ohio, which is the leading variety at present in the lower Corn Belt, seems much more subject to second growths of the tubers than other varieties grown at Columbia the past four years. Perhaps such varieties as the Irish Cobbler, which has little tendency to second growth, would react quite differently if subjected to spraying treatment under the same conditions as those mentioned in the test with the Early Ohio variety. If the increase in yield can be secured, without distortion of the tubers,

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on satisfactory early sorts such as the Irish Cobbler, spraying should be a profitable practice in this region.

FIG. 1. Effect of Bordeaux mixture on vitality of Irish potato plants. 2 rows on right unsprayed. On left, sprayed four times with Bordeaux mixture 4-4-50 alone, also the same with arsenate of lead, and with arsenate of lead plus nicotine sulphate. Photographed at Columbia, Missouri, July 25, 1921.

During the growing season the past year, the weather alternated, with short wet periods interspersed between longer hot, dry spells. The result, of course, was a sharp variation in soil moisture in the potato field. It occurred to the writer that such soil-moisture fluctuations might be the immediate cause of the second growth on the potato tubers, especially on those from the sprayed plots, which continued in vigorous growing condition during that portion of the summer when the soil-moisture fluctuations were sharpest. Furthermore, since Duggar and Bonns (2) have shown that a film of Bordeaux spray on the leaves of the potato plant increases the transpiration rate, it may be supposed that the water deficit is greater in sprayed plants in the field during dry periods. This notion appears all the more probable when the larger expanse of leaf surface possessed by the sprayed plants is considered. Presumably, tuber growth ceases when lack of moisture makes conditions unfavorable for the development

of the plant as a whole. When rain comes, growth again proceeds vigorously for a few days. The tuber development resulting from such spurts of growth, in the case of the Early Ohio variety, seems to consist very largely in knobby protuberances from the eyes, especially at the tips of

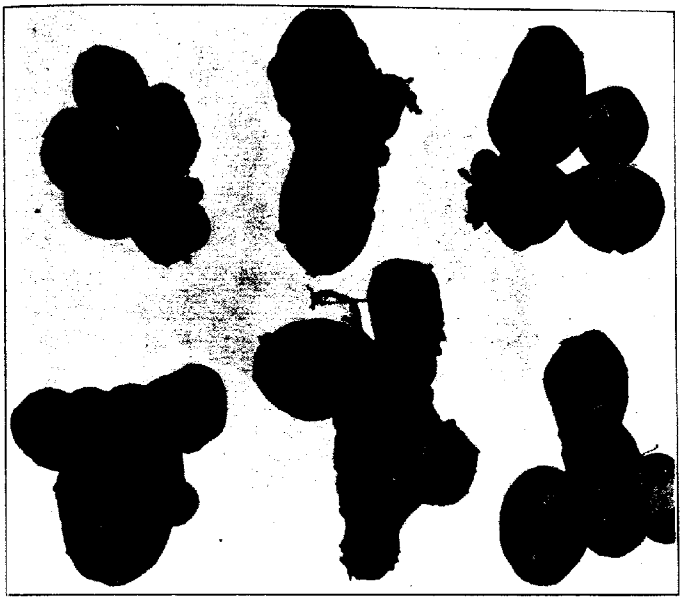

FIG. 2. Knotty protuberances or second growths on tubers of early Ohio potatoes from plots sprayed four times with Bordeaux mixture.

tubers previously formed. As many as four or five distinct growth zones could be identified on some of the knotty tubers from the sprayed plots. It is hoped that further experiments can be carried out to learn the limits of variation in soil moisture which the potato will endure without developing second growths, as well as the relation of certain other edaphic factors to tuberization. The state of dormancy in tubers whose growth is checked as above indicated is also a question of some interest, for if removed from the plant these tubers would not commence stem growth for many weeks.

Another interesting question suggested by the observations described above is the value of tubers bearing second growths for seed purposes. If variation in soil moisture or in some other environmental factor causes tubers to develop second growth in Missouri, this defect may arise from the same causes in Wisconsin, Minnesota, or other seed-growing section. Then tubers bearing second growth would not necessarily be weak or de-

fective in any way physiologically, and there need be no discrimination against such tubers for seed purposes, although such discrimination has been customary. In fact, the experiments of Dr. Salaman (4) in England showed that knobby second growths planted as seed-pieces produced a larger crop than pieces from normal tubers, and that the tendency to second growth was not transmitted. This suggests another question, then, as to the advisability of submitting to critical tests some current ideas concerning standards of quality in Irish potatoes, at least from the standpoint of seed growing and plant improvement.

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# INTERNAL DECLINE OF LEMONS

## II. GROWTH RATE, WATER CONTENT, AND ACIDITY OF LEMONS AT DIFFERENT STAGES OF MATURITY [1]

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This work was undertaken to determine the possible bearing on the etiology of internal decline of lemons [2] of (*a*) the rate at which lemons increase in size as influenced by climatic and seasonal changes and by the time of year at which the fruit is set, and (*b*) the increase in acidity and water content of the fruits at different stages in their development.

Statements have been made by different writers concerning the acidity and water content of mature lemons but not of lemons at different stages of maturity. Wehmer (8) gives the water content of mature lemons as 82.64 percent, a figure which is too low, at least for California lemons, as is indicated later in this discussion. Clark and Lubs (2) give a pH value of 2.2 for the true acidity of juice from mature lemons. The results obtained in this work agree very well with that figure.

### Methods of Experimentation

Groves located at Corona, Upland, and Riverside were chosen as suitable places in which to carry on the work. These groves are all of different ages, as follows: at Riverside, 6 years; at Corona, 20 years; and at Upland, 30 years. All three groves are Eurekas, the variety usually grown in California.

The sizes of the lemons were determined by diameter measurements with a vernier caliper. In each of the three groves 200 small lemons were measured and tagged each month for one year. When a new lot was measured and tagged, those previously tagged and measured were remeasured, so that each lemon was measured each month until it dropped from the tree or until it came to maturity and was picked. Fruits were tagged on a chosen group of trees and in many different locations on each tree. Fruits averaging from 0.44 to 1.29 cm. in diameter were chosen for the initial measuring and tagging. Fruits of these sizes are about 1 to 2 months old, depending upon the season and other conditions attending their growth. Each month, following the month of initial measuring and tagging, samples were brought to the laboratory and tested for acidity and water content.

[1] Paper no. 95, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] For a description of the abnormality known as internal decline of lemons, the reader is referred to the first article in this series by Bartholomew, Barrett, and Fawcett (1).

In making the tests for acidity and water content, the lemons were cut once longitudinally and once transversely, thus dividing them as nearly as possible into 4 equal parts. One half of each end was used for the acidity test and the other half to determine the water content. This was done to determine whether or not there were any differences between the acidities and water contents of the two ends of the lemon. In determining the acidity the fleshy pulp alone of the fruit was used, but both pulp and peel were used in determining the water content.

The hydrogen electrode was used to determine the acidity. The pulp to be tested was cut free from the peel and ground in a mortar, and the juice was extracted in a tincture press. In making the water-content determinations the peel and pulp were finely cut, placed in weighing bottles, and dried to constant weight in a vacuum oven.

In connection with the determinations of acidity of lemons at different stages of growth, other tests were made to determine the acidity of mature lemons taken from the packing houses or directly from the trees. A series of experiments also was performed to determine whether or not the leaves of the lemon trees may be an active factor in drawing water from the fruits.

## Results and Discussion

### Growth Rate and Water Content of Lemons

The subjects of growth rate and water content are so closely related that no attempt will be made to discuss them separately.

The Eureka lemon tree is one which, under conditions of comparatively high humidity and low temperature, such as prevail near the coast in southern California, produces new fruit in practically every month of the year. Farther inland, where the humidity is lower and the temperature higher, there is a more marked tendency toward seasonal production. In this respect, fruit production apparently is influenced also by the age of the tree, because the older the tree the greater tendency it shows toward seasonal rather than continuous production. Evidence of this latter condition was shown in the three groves chosen in which to carry on this work. It was found that no fruits were set during the month of September at Riverside where the trees were 6 years old; none during September and March at Corona where the trees were 20 years old; and none during August, September, October, and March at Upland where the trees were 30 years old.

It was found also that, although there is a tendency toward continuous production of fruits, many more are produced during the spring set of fruit than at any other time of the year. This agrees with the findings of Reed (6) in his study of the relation between the flowers and the fruits of the lemon. He found that approximately 66 percent of the fruit buds appear during March and April, 17 percent from May to October inclu-

sive, 13 percent in November, and about 3 percent from December to February inclusive.

The month of the year in which fruits are set determines to a certain extent whether they will soon drop off or will remain on the tree till they mature. The results of the experiment as carried on in these three groves indicate that, at least under climatic conditions such as prevailed in this region from June 9, 1920, to August 9, 1921, fruits set in April, May, and June have the best chance of reaching maturity. Climatic and soil conditions and the time of year at which fruits are set are important factors in determining whether or not they will remain on the trees till mature. This is shown by the fact that of some of the lots of 200 lemons tagged, practically 100 percent remained on the trees, while of some of the other lots as much as 50 or 60 percent, and in one or two cases even 90 percent, fell off before they were more than 2 months old. That the mortality is often very high is indicated perhaps more strikingly by Reed's (6) work, which shows that out of 4,440 flower buds, 51.98 percent set fruits, 21.71 percent reached a diameter of ¼ inch, and only 6.62 percent reached maturity.

It was found that the growth rate of individual lemons is influenced not only by soil and climatic conditions and the time of the year when set, but also by the location on the tree. While the height on the tree and the location, whether within the foliage or exposed on the outside, appeared to have an influence, the most important factor seemed to be the condition of the branch on which the fruit was borne. While this is true also for deciduous fruits, it appears to be more marked in the case of lemons. This variation is probably due to differences in the amounts of water and food substances carried by the different branches. It was found, for example, that some of the fruits set in April were ready to be picked in the following October and November, a period of 7 and 8 months after being set, while other lemons on the same tree were not up to picking size for at least 14 months after being set.

It is impossible to present here all the interesting data obtained in these experiments on growth rate, water content, and acidity of lemons. The figures given in table 1 are a summary of the data obtained in one of the series in the grove at Corona. This is a fair example of all the data secured in all the series. The lemons in this series were first measured and tagged August 8, 1920. The average diameter when tagged was 1.15 cm.

It will be seen by reference to table 1 that the growth rate of the lemons was fairly constant from August 8 to February 10, during which period the lemons increased in size from 1.15 to 4.60 cm. However, on the date of the next measurement, March 9, the average size of the lemons had decreased from 4.60 to 4.53 cm. Further observation will show that another slight decrease took place from June 9 to July 8. A glance at the figures in the columns headed "water content" will show that at the time of this last

decrease in size of the lemons there was a corresponding decrease in the water content. While there was not an actual decrease in water content from February 10 to March 9, yet the increase was very small, far from commensurate with previous increases.

TABLE 1. *Growth rate, water content, acidity, and color of lemons at different stages of maturity*

| Date, 1920 and 1921 | Average Diameter (cm.) | Percent, Each Color | | | pH Value | | Water Content (percent) | |
|---|---|---|---|---|---|---|---|---|
| | | Green | Silver | Yellow | Stylar End | Stem End | Stylar End | Stem End |
| Sept. 10 | 2.17 | 100 | | | 4.46 | 4.46 | 53.97 | 53.68 |
| Oct. 9 | 2.86 | 100 | | | 2.91 | 3.08 | 75.42 | 74.50 |
| Nov. 10 | 3.34 | 100 | | | 2.64 | 2.71 | 81.45 | 81.61 |
| Dec. 10 | 3.63 | 100 | | | 2.54 | 2.50 | 83.03 | 82.16 |
| Jan. 10 | 3.91 | 68.1 | 31.9 | | 2.60 | 2.64 | 84.01 | 84.21 |
| Feb. 10 | 4.60 | 18.9 | 75.8 | 5.3 | 2.57 | 2.54 | 85.57 | 85.69 |
| Mar. 9 | 4.53 | 11.4 | 64.5 | 24.1 | 2.33 | 2.36 | 85.74 | 85.93 |
| Apr. 11 | 4.73 | 6.8 | 71.6 | 21.6 | 2.30 | 2.33 | 86.73 | 86.72 |
| May 9 | 4.92 | 4.8 | 76.2 | 19.0 | 2.23 | 2.27 | 88.99 | 88.59 |
| June 9 | 5.18 | One Lemon | 73.0 | 25.1 | 2.29 | 2.33 | 89.99 | 88.82 |
| July 8 | 5.16 | One Lemon | 71.2 | 26.4 | 2.29 | 2.33 | 88.20 | 87.30 |
| Average | | | | | 2.65 | 2.69 | 82.10 | 81.75 |

The facts that on the dates of two measurements, March 9 and July 8, the lemons were actually smaller than at the time of the preceding measurements, and that these decreases in size were accompanied on March 9 by only a very slight increase in water content and on July 8 by an actual decrease, raise the interesting question as to what may have been the cause of these conditions. The results of the following experiments give a direct answer to this question.

1. Lemon branches about 45 cm. long, each bearing from 1 to 3 mature or nearly mature lemons, were brought into the laboratory. The lemons were detached from half of the branches while the remaining branches were left as brought from the grove. The detached lemons, the branches from which they had been taken, and the branches bearing lemons were all placed on the laboratory table. At the end of 24 hours it was found that the detached lemons, as nearly as could be detected by the touch, were firm and in normal condition while the leaves on the branches from which these lemons had been taken were wilted and drooping. On the other hand, the leaves on the branches to which the lemons were still attached retained their luster and were normally rigid and upright while the lemons on these branches had become quite soft to the touch.

Further experimentation showed that the leaves on branches from which the lemons had been detached showed signs of wilting within a very few

hours, while the leaves on branches to which the lemons were still attached remained turgid and upright for thirty-five hours or more. The length of time depended, of course, upon the number of leaves per fruit and the humidity and temperature of the surrounding air.

2. Small branches, each bearing about 8 or 10 leaves and one lemon, were chosen for the next experiment. A thin slice was cut from the stylar end of each lemon and the cut end was immersed in eosin. The remainder of the lemon and the branch were left exposed to the air. Examinations were made at intervals. It was found that by the end of 12 to 15 hours the pull of evaporation had drawn the eosin up through the lemon and out into all the leaves.

3. Experiments were performed to determine the comparative amounts of water lost by detached lemons as compared with those remaining attached to the severed branches. No attempt was made to control the temperature or humidity conditions. Determinations were made after the detached lemons and the lemons still remaining attached to the branches had remained under ordinary laboratory conditions for certain lengths of time. At the expiration of a given time the entire lemons were cut fine, placed in weighing bottles, and dried in a vacuum oven. The results showed that for any given time the lemons that were still attached to the branches lost 12 to 15 times as much water as those which had been detached from the branches. Experiments similar to numbers 1, 2, and 3 were performed by Hodgson (4) on the navel orange with the same general results.

4. The fact that lemons allowed to remain attached to branches cut from the tree will lose water much more rapidly than lemons detached from the branches was further proved by the freezing-point method. Two branches were taken from each of 10 trees. These branches were divided into 2 lots in such a manner that each lot contained one branch from each tree. Each branch bore one lemon about 4.45 cm. in diameter. The lemons from one set of branches were at once detached and prepared for making the freezing-point determination. The other set of branches, with the lemons still attached, was placed in an oven at 46° C. for 4½ hours. At the end of this time these lemons were taken from the branches and treated as in the case of the first lot. In preparing the lemons for the freezing-point test one third of each end of each lemon was used. Both peel and pulp were ground in a meat grinder, and the juice was extracted in a tincture press. In order to get a check on this method of treatment the experiment was repeated in all details except that the second lot of lemons was placed in the oven for 4½ hours at 46° C. after they had been detached from the branches. An average of ten lemons was used in each trial.

A summary of the results of this experiment is given in table 2.

TABLE 2. *Comparative losses of water from attached and detached lemons as shown by the freezing-point method*

| No. of Test | Nature of Tests | | Mean Δ | Mean Concentrations (atmospheres) |
|---|---|---|---|---|
| 1 | Lemons detached from branches at once.. | Stylar end. | 1.077 | 12.96 |
| | | Stem end.. | 1.062 | 12.79 |
| | Lemons detached from branches *after* being in oven 4½ hours at 46° C........ | Stylar end. | 1.193 | 14.35 |
| | | Stem end.. | 1.198 | 14.37 |
| 2 | Lemons detached from branches at once.. | Stylar end. | 1.182 | 14.22 |
| | | Stem end.. | 1.150 | 13.84 |
| | Lemons detached from branches *before*.. being placed in oven 4½ hours at 46° C... | Stylar end. | 1.192 | 14.35 |
| | | Stem end . | 1.196 | 14.39 |

The results in the first half of table 2 show that there was a marked increase in the concentration of the juice in the lemons that were in the oven for 4½ hours, the increase being 1.39 atmospheres for the stylar end and 1.58 atmospheres for the stem end. That this increase in concentration could not have been due, to any marked extent, to water passing out through the peel of the lemons is shown by the figures in the latter half of the table, for here the stylar end shows a difference of only 0.13 atmosphere and the stem end one of only 0.55 atmosphere. The marked difference between the first and second tests is due to the fact that the first was made about 6 weeks earlier than the second one. At the time of making the earlier test the trees were still growing, and the determinations were made after irrigation and a light rain followed by heavy dews at night. This, of course, would tend to make the fruits more turgid, and hence to reduce the sap concentration.

5. The results of another experiment are offered as further proof that the leaves may draw water from the fruits. Branches about 60 cm. long, each bearing from 1 to 3 lemons, were cut from the trees and at once cut again under water. These branches were then brought to the laboratory, placed with the cut ends in jars of water, and all set in a well-ventilated oven heated by electricity. During the day the oven temperature ranged from 35 to 38° C. About 5 p.m. the heat was turned off and not turned on again until about 8:30 a.m. the next day. The temperatures at night ranged from about 15 to 18° C. The leaves on the branches under these conditions retained a normal appearance for about 5 to 7 days. At the end of this time they began to wilt and lose their luster. By the end of 8 to 10 days the leaves began to drop. About this time the lemons were taken from the branches and thin slices were cut from the stylar ends, the knife passing through the point of juncture of pulp and peel. Observation of the cut surfaces showed that in a large number of the lemons, though not

in all, the tissue had broken down, thus leaving small circular openings about 1 mm. in diameter adjacent to each main vascular bundle. These openings have been termed "drying-out holes." Cutting of control lemons at the time these branches were taken from the tree showed that these lemons were in a normal condition at the beginning of the experiment. Thousands of lemons taken from dry portions of groves and cut have shown these drying-out holes, while comparatively few or none of the lemons from the more moist portions of the same groves showed them. The variation in moisture content in different parts of a grove may be due to such factors as differences in soil texture or an uneven distribution of irrigation water.

That there must be some relation between these drying-out holes and the appearance of internal decline is indicated by the fact that practically every lemon having decline shows these holes when it is cut for examination. This experiment coupled with the field observations indicates that this collapse of tissue, caused by the withdrawal of water from the fruit, is the first visible step in the production of internal decline.

The results of the preceding 5 experiments show very clearly that the lemon fruits act as water reservoirs for the leaves. When the roots fail to supply adequately the demand of the leaves for water, the leaves begin to draw it from the fruits. In a climate such as exists in the inland lemon-growing districts of California, the leaves will begin to draw water from the fruits even before the soil moisture has become very materially depleted. On going into a grove in the afternoon on a warm summer day when the temperature is near 100° F. and the psychrometer shows a humidity of 20 percent or less, the lemon fruits are found soft to the touch even though the soil moisture content may be well above the wilting coefficient *of the leaves.* Upon examination of the same lemons the following morning they will be found turgid. This condition becomes especially noticeable when there is a wind blowing, since this increases the rate of evaporation from the leaves. The withdrawal of water from the fruits by the leaves is an important factor in irrigated districts such as this, because, if the water supply in the soil becomes too low, or if climatic conditions remain unfavorable too long, not only the fruits but also the leaves may be caused to fall from the trees.

In connection with the foregoing statements it might be well to emphasize the fact that, at least in the case of such plants as citrus trees, which may retain their fruits from several months to a year or more, the term "wilting coefficient" of the soil, as usually applied, has little significance. There can be no doubt that water may be withdrawn from the fruit to an injurious extent by the leaves long before there is any sign of wilting in the leaves. For such plants as the citrus fruits it would be interesting and profitable to have determined a wilting coefficient based on fruit rather than on leaf conditions. However, this would probably be difficult except as a coefficient might be worked out for each individual kind of fruit.

These experiments proving that water may be withdrawn from the fruits by the leaves are not given as proof of a new discovery. They are reported (*a*) because it appears that the importance of this phenomenon of water-withdrawal from the fruits by the leaves is not generally realized, (*b*) because of their bearing on the major experiments reported in this article, and (*c*) because of their bearing on our practice of growing citrus and similar plants under irrigation in a semi-arid region, rather than in their native habitats which have abundant rainfall and high humidity. Further discussions bearing directly or indirectly on this subject of withdrawal of water from the fruits by the leaves may be found in articles written by Renner (7), Livingston and Brown (5), Coit and Hodgson (3), Hodgson (4), and others.

By referring to table 1 it will be seen further that there is very little difference between the water contents of the two ends of the lemon. This series shows an average of 0.35 percent more water in the stylar end than in the stem end. Some of the other series show even less difference than this series.

It was found during these experiments that the water content of young fruits is affected by the availability of water. For example, in September, when the water supply was limited and relative evaporation was high, the water content of lemons 1.9 cm. in diameter averaged 54 percent. In December, when both rain and irrigation water were available and the amount of evaporation was comparatively small, the water content of lemons only 1.27 cm. in diameter averaged 68 percent. The variation of water content as affected by availability of water and climatic conditions was noticed also in the mature lemons. The variation may be as great as 10 percent or more, but it is usually less. The range of differences of water contents in the mature fruits tested from the three experimental groves was from 88.20 to 92.14 percent. Wehmer (8) reports only 82.64 percent for mature lemons.

Further reference to table 1 will show that the increase in water content is comparatively small after the lemon has attained an average diameter of about 3.8 cm.

During the course of these experiments it was noted that there were two periods in the year when there was a tendency toward cessation of growth. One occurred during the colder months and probably was due to climatic conditions that caused a decrease in metabolic activity and a tendency toward a normal rest period. The other occurred during the hottest months of the year and in all probability was due to low humidity and insufficient soil moisture. It is quite often the case in lemons that are approaching maturity that, when growth begins again after being checked, it occurs almost wholly in the peel, thus making a lemon with a thick peel which is undesirable for marketing.

## Acidity

It was found in studying the acidity of the lemons that, while the total acid content increases as the size of the lemon increases, the true acidity of the juice, like the water content, increases comparatively little after the lemon has reached a diameter of about 3.8 cm. By referring to table 1 it will be seen that in this series, lemons averaging 3.63 cm. in diameter showed average pH values of 2.54 for the stylar end and 2.50 for the stem end, while 5 months later, when the lemons in this same series had attained an average diameter of 5.18 cm., the pH values were only 2.29 and 2.33. In another series, when the average diameter was 4.32 cm., the pH values were 2.57 and 2.54. Eight months later the average diameter had increased to 5.72 cm., but the acidities had increased only to pH 2.33 and 2.36. These two examples are typical of all the series.

Lemons which are all approximately of the same age and size show a great deal of individual variation in acidity. For example, mature lemons taken from storage and tested individually showed variations in acidity such as the following: pH 2.27, 2.40, 2.37, 2.29.

The average acidity obtained for all the mature lemons tested was pH 2.31. This value is somewhat lower than it would have been had the tests been made on first-class market lemons. Some of the lemons tested in this work had been allowed to remain on the trees until they were in the condition known to the growers and packers as tree-ripe.[3] When lemons have reached this condition they have a slightly higher sugar and slightly lower acid content than earlier.

Variations in acidity were found not only in individual lemons but in the different ends of the same lemons. In some lemons it was the stylar end and in others the stem end which showed the higher acidity. This may be seen in table 1, and in the figures given in the second paragraph preceding this one. However, the tests, when averaged, showed practically no difference between the acidities of the stylar and stem ends of over 400 normal lemons. The average of the total number of tests showed the stem end to be pH 0.01 more acid than the stylar end. This difference is so small, however, that it comes well within the limits of experimental error.

## Summary

The principal results obtained by the experiments on growth rate, water content, and acidity of lemons at different stages of maturity may be summarized as follows:

1. While the lemon tree tends toward the production of new fruits continuously, the age of the tree and climatic and soil conditions make the production more or less seasonal. In the inland districts the seasonal setting of new fruits is more marked than in the coastal regions.

[3] For an explanation of the term "tree-ripe" and similar terms referring to the stages of maturity of lemons, see the first article in this series by Bartholomew, Barrett, and Fawcett (1).

2. The time of the year when set, the age of the tree, and climatic and soil conditions are all factors determining the growth rate of the fruits. Some fruits may mature in 7 or 8 months, while others growing on the same tree may require as much as 14 months in which to mature.

3. Lemons may actually decrease in size while still attached to the tree, in consequence of the withdrawal of water from them by the leaves. This withdrawal of water from the fruits by the leaves may result in the collapse of at least a portion of the tissue in the stylar end of the fruit.

4. The wilting coefficient of the soil as indicated by lemon leaves can not be considered a safe criterion as to whether or not the lemon fruits are suffering from a lack of water.

5. There is practically no difference between the water contents of the two ends of the normal lemon.

6. As the lemon enlarges, its water content increases, but this increase is much more rapid up to the time that it is about 3.8 cm. in diameter than from that time to maturity.

7. The size of the lemon is not necessarily proportional to the percentage of water it contains. In September a lemon 1.90 cm. in diameter may have a much lower water content than a lemon 1.27 cm. in diameter in December.

8. Mature lemons may show considerable variation in water content. The range in this series of experiments was from 88.20 to 92.14 percent.

9. While the total acid content of the lemon increases rapidly as it approaches maturity, the true acidity increases very little after the lemon has reached a diameter of about 3.8 cm.

10. There are quite wide variations, but the average of a large number of stylar and stem ends of normal lemons shows the mean acidity to be substantially the same for each.

11. Mature lemons of practically the same age and size have a comparatively wide range of acidity.

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# INFLUENCE OF TEMPERATURE ON THE PECTINASE PRODUCTION OF DIFFERENT SPECIES OF RHIZOPUS

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Investigations (1) have shown that the following species of Rhizopus with the exception of the last two are parasitic on the sweet potato: *nigricans* Ehrnb., *reflexus* Bainier, *tritici* Saito, *artocarpi* Racib., *delemar* (Boid.) Wehmer and Hanzawa, *maydis* Bruderl., *nodosus* Namysl., *oryzae* Went and Pr. Geerligs, *arrhizus* Fischer, *microsporus* v. Tieg., and *chinensis* Saito. These fungi produce an enzym which dissolves out the middle lamellae so that the cells lose their coherence, thereby transforming the potatoes into a soft, watery mass. The cells themselves, however, are not penetrated, at least in the early stages of decay. It was likewise shown (2) that the two nonparasitic as well as the parasitic species produce an enzym, a part of which is exuded into the substrate which macerates raw sweet-potato disks in from two to four hours. The nonparasitic species actually produce more enzym than some of those which cause decay of sweet potatoes. Furthermore, it was found that the maximum strength of the macerating enzym both in the solution and in the mycelium is reached after a two or three days' growth of the organisms in sweet-potato decoction.

Harter, Weimer, and Lauritzen (1) showed that these species of Rhizopus could be roughly grouped into high-, medium-, and low-temperature forms. The results demonstrated that a low temperature is not so much a limiting factor to infection and decay as a high temperature, since intermediate forms produced decay under laboratory conditions at relatively low temperatures, while the low-temperature forms did not infect sweet potatoes at a temperature of 30° C. or above.

In view of the relationship found to exist between the different species with respect to the temperature at which they will infect and decay sweet potatoes, the writers proposed to determine (1) if pectinase is produced at any temperature at which the fungus will grow, and (2) if the amount produced is greatest at the optimum temperature for infection and decay.

## METHOD OF EXPERIMENTATION

The methods employed are, for the most part, the same as those previously described by the writers (3), but modified when necessary to meet the requirements of the problem. The fungi were grown on sweet-potato decoction in 2-liter Erlenmeyer flasks held in incubators maintained at constant temperatures of 9°, 20°, 30°, and 40° C. The organisms were

grown at the three higher temperatures for 3 days and at the lower for 26 days. A luxuriant growth resulted in 3 days at 20°, 30°, and 40° C., thereby furnishing a sufficient amount of mycelium for enzymic studies. However, at 9° barely enough material for this purpose was produced after 26 days of growth. The decoction in the two flasks upon which the same species had grown was combined into one compound sample, and aliquot parts were taken for maceration experiments. The solution was handled and the mycelium treated according to a method previously described (3). Maceration was regarded as complete when the disks offered no resistance when pulled from opposite directions. The enzymic action of the mycelium was determined by suspending 0.50 g. of pulverized material in 25 cc. of distilled water, in accordance with methods previously described (3).

The raw disks (1.5 mm. in diameter and 0.5 mm. thick) which were employed to measure the macerating power of the enzym in each experiment were cut from within the fibro-vascular ring of one sweet potato. The experiments with all the organisms were repeated several times at each temperature. Flasks in which the enzym was inactivated by steaming, as well as flasks containing some of the original decoction which had not been inoculated, were employed as controls in all the experiments. The sweet-potato decoction was made in sufficient quantity of uniform composition for one entire experiment with all the species.

Maceration was carried out at 40° C. regardless of the temperature at which the organisms were grown. The solutions, previously filtered through cotton to remove the fungous material, were brought to the temperature at which maceration was to take place by exposing them for one hour at 40° C. before the raw disks were added. After the disks were added to the solutions they were examined at frequent intervals in order to study the progress of maceration.

## Experimental Data

The results of the various experiments with each organism at the different temperatures have been averaged and set forth in table 1. The length of time required to macerate the raw disks completely is given in hours. It is hardly necessary to point out that any method no more refined than the one employed here is certain to give some variation in the results. Some of the factors which have an influence on the results will be discussed later.

Table 1 shows that at only one temperature (20° C.) were data obtained on the maceration of the raw disks by all the organisms. At 40° and 30° the species *microsporus* and *nigricans* made no, or at least such a feeble, growth in the time allowed that neither the solution nor the mycelium contained a measurable quantity of pectinase. Likewise certain species, namely, *delemar*, *chinensis*, and *maydis*, made no growth at 9° in 26 days, the time allowed for development at the lower temperature. A small

amount of enzym was exuded into the substrate by *reflexus* at 40° and its action was determined, but the actual amount of mycelium produced was so small that its macerating power could not be measured.

TABLE I. *Average rate of maceration by the enzym exuded into the solution upon which the different species of Rhizopus grew at various temperatures, as well as by that retained by the mycelium**

| Temperature for Growth of Fungi (° C.) | *R. reflexus* | *R. delemar* | *R. oryzae* | *R. chinensis* | *R. nodosus* | *R. tritici* | *R. microsporus* | *R. maydis* | *R. nigricans* |
|---|---|---|---|---|---|---|---|---|---|
| In Solution | | | | | | | | | |
| 40 | 10 | 4.7 | 4.7 | 4.9 | 3.25 | 4 | ....... | 5.5 | ....... |
| 30 | 2.75 | 2.1 | 2.15 | 4.5 | 2.05 | 1.45 | ....... | 1.3 | ....... |
| 20 | 2 | 2 | 1.5 | 5 | 1.75 | 1.25 | 1.75 | 1.25 | 4.87 |
| 9 | 2.5 | ....... | 3.25 | ....... | 1.87 | 2 | 24+ | ....... | 48+ |
| In Mycelium | | | | | | | | | |
| 40 | ....... | 8 | 8 | 8–24 | 4 | 4.5 | ....... | ....... | ....... |
| 30 | 3 | 5 | 3.8 | 6 | 3.8 | 2.5 | ....... | 2.5 | ....... |
| 20 | 3 | 3 | 3.5 | 6.5 | 3 | 2.5 | 8 | 3 | 8–24 |
| 9 | 2.75 | ....... | 2.5 | ....... | 2.87 | 2.67 | 24 | ....... | 24 |

* Figures indicate time in hours necessary to complete maceration.

The table shows that the enzym content of the solution was least when the different species were grown at 40° C., the time required to macerate the disks varying from 3.25 hours to 10 hours. *Reflexus*, a low-temperature form, made a very feeble growth at 40°. Under these conditions the total amount of pectinase produced by it was small in comparison with that secreted by the other species grown at the same temperature. On the other hand, the time required to macerate the disks was considerably shortened when the growth took place at 30°. This is particularly true of *reflexus*, which grew much better at the latter temperature. With respect to this temperature, it is interesting to note that *chinensis*, which, together with *microsporus*, was shown (1) not to be parasitic on sweet potatoes, required a much longer time to macerate the tissue than any of the other species. Both species, however, were shown elsewhere (2) to produce a pectinase when grown at 30° which would disintegrate raw sweet-potato tissue. At 20° all the species made a good growth and exuded a considerable quantity of enzym into the substrate. The time required for the enzym to complete maceration was less when the fungus was grown at this temperature than when it was grown at 30°, with one exception, *chinensis*, although the difference in time here is not great. Both *chinensis* and *nigricans* required two to four times longer than any of the other species completely to dissolve out the middle lamellae of the disks. At 9° several of the fungi made no growth. This is particularly true of the high-, and of some of the intermediate-temperature forms. The enzym exuded into the solution by *microsporus* and *nigricans*, which made a slight growth at 9°, required 24

and 48 hours, respectively, to complete maceration. A comparison of these two species when grown at 9° and 20° shows a decided decrease in the amount of pectinase produced at the lower temperature, although both are relatively low-temperature forms. The losses caused by the latter species frequently occur at a temperature not much higher than the lowest temperature used in these experiments. The tissues of potatoes decayed by *nigricans* under storage conditions are completely disintegrated, and the middle lamellae are dissolved out so that coherence is entirely lost, a fact which suggests that a pectinase is produced. Furthermore, the decay is rapid, and the dissolution of the middle lamellae takes place considerably in advance of the growth of the hyphae.

A study of the data derived from the use of the mycelium shows some interesting facts. It should be pointed out in this connection that the rapidities of maceration by the enzym in the solution and in the mycelium are not strictly comparable, since no attempt was made to use a quantity of enzym powder that would be equivalent in macerating power to the enzym contained in the solution. It will be seen that the mycelium of *oryzae*, *delemar*, and *chinensis* grown at 40° C. contained a small amount of enzym. *Microsporus*, one of the nonparasitic species, produced a small amount at 20° and at 9°. A comparison by this method of *chinensis* and *microsporus* with *nigricans*, a species which readily decays potatoes at 20° or lower, indicates that the latter produces a smaller amount of the macerating principle. The comparison of the results obtained with *microsporus* and *nigricans* in the solution at the same temperature shows similar results. There are some outstanding differences between the results obtained with the solution and those obtained with the mycelium. It has been pointed out that the amount of enzym in the solution increased with the decrease of temperature from 40° to 20°, and then decreased when the temperature was lowered to 9°. On the other hand, results obtained from the mycelium did not follow the same general course when the temperature was lowered from 20° to 9°. As a matter of fact, the results here show that, with one or two exceptions, there is more pectinase in the mycelium grown at 9° than in that grown at 20°. It is interesting to note in this connection that Harter (4) found amylase to be present in larger amount in the mycelium of *R. tritici* grown at 9° C. than at any higher temperature tried up to 40° C. On the whole, the results show that a larger amount of pectinase is produced per unit measure at intermediate to relatively low temperatures than at high ones. In two cases in which mycelium was used, *chinensis* at 40° and *nigricans* at 20°, the results are recorded in the table as varying from 8 to 24 hours. This merely means that some maceration had started in 8 hours, and that the process was completed in 24 hours, no further examination of the material having been made.

The results of these investigations show that, at least within the limits of these experiments, pectinase is produced at any temperature at which the organism grows. The amount produced is least at the higher and most

at the intermediate temperatures or in the vicinity of 20° C. Since this temperature is favorable for decay, it may be assumed that, within rather wide limits, the amount of pectinase produced is greatest at the temperature most suitable for the decay of sweet potatoes.

## Maceration of Old and New Potatoes

Some of these investigations were conducted during the summer and autumn months, when either old sweet potatoes kept from the preceding crop or immature potatoes taken from the ground had to be used. The question naturally arose whether the old and the new potatoes would be macerated at the same rate.

It is believed by some investigators that sweet potatoes which have been in storage for some time are more readily decayed than those freshly dug, and there is some evidence which indicates that such is the case. The writers therefore decided to carry out a series of experiments to determine if there is any difference in the rate at which the middle lamellae of stored and freshly dug potatoes are dissolved. The experiments were conducted according to the method already described with all the species of Rhizopus, the organisms having been grown at 40°, 30°, and 9° C. and maceration carried out at 40°. At 40° and 30° the organisms were grown for 3 days, at 9° for 26 days. The old potatoes had been in storage for about 10 months. The new potatoes were freshly dug or had been in storage for only a few weeks at most.

The results of these investigations are shown in table 2.

TABLE 2. *Comparative rate of maceration of old and new potatoes**

| Temperature for Growth of Fungi (° C.) | Kind of Potatoes | *R. reflexus* | *R. delemar* | *R. oryzae* | *R. chinensis* | *R. nodosus* | *R. tritici* | *R. microsporus* | *R. maydis* | *R. nigricans* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Solution | | | | | | |
| 40 ........ | Old...... | 10 | 4.7 | 4.7 | 4.9 | 3.25 | 4 | ...... | 5.5 | ...... |
| | New...... | 12 | 5.25 | 5.5 | 12 | 3.75 | 4.5 | ...... | 7 | ...... |
| 30 ........ | Old...... | 2.75 | 2.1 | 2.15 | 4.5 | 2.05 | 1.45 | ...... | 1.3 | ...... |
| | New...... | 4.5 | 4.25 | 4.5 | 6 | 4.25 | 2.75 | ...... | 2 | ...... |
| 9 ........ | Old...... | 2.5 | ...... | 3.25 | ...... | 1.87 | 2 | 24+ | ...... | 48+ |
| | New...... | 3 | ...... | 5 | ...... | 2.4 | 2.5 | 48+ | ...... | 48+ |
| | | | | Mycelium | | | | | | |
| 40 ........ | Old...... | ...... | 8 | 8 | 8-24 | 4 | 4.5 | ...... | ...... | ...... |
| | New...... | ...... | 7 | 7 | 14 | 3.5 | 4.5 | ...... | ...... | ...... |
| 30 ........ | Old...... | 3 | 5 | 3.8 | 6 | 3.8 | 2.5 | ...... | ...... | ...... |
| | New...... | 3.25 | 5.75 | 5.25 | 14 | 5 | 3.25 | ...... | ...... | ...... |
| 9 ........ | Old...... | 2.75 | ...... | 2.5 | ...... | 2.87 | 2.67 | 24 | ...... | 24 |
| | New...... | 4 | ...... | 4 | ...... | 4 | 4 | 48+ | ...... | 48+ |

* Figures indicate time in hours necessary to complete maceration.

The results show that in nearly all cases the old potatoes are more readily macerated than the new. In some instances, for example, *chinensis*,

a nonparasitic species, the difference is quite marked. Unfortunately, some of the organisms would not grow at 40° and 30° C. and others at 9°, at least within the time allowed. It is evident from table 1 that *nigricans*, the principal cause of soft-rot of sweet potatoes, produces a very small amount of pectinase at 9°. The conclusion from the results shown in table 2 is that potatoes which have been cured and kept in storage for several months are more readily macerated than those freshly dug, the difference in most cases being very marked. The time required to macerate old potatoes is on an average about one half that required to dissolve the middle lamellae of new ones, as regards both the enzym exuded into the substrate and that retained by the mycelium. These results would seem to accord with the general observation that the susceptibility of sweet potatoes to decay increases with the increase in length of time they are held in storage.

## Summary

1. The influence of temperature on pectinase production by the following species of Rhizopus was studied: *nigricans, reflexus, microsporus, delemar, oryzae, chinensis, nodosus, tritici*, and *maydis*. These experiments seem to indicate that the enzym is produced at any temperature at which the fungi will grow. Temperatures of 9°, 20°, 30°, and 40° C. were employed.

2. The amount of enzym produced was least at the highest temperature, as regards both that exuded into the substrate and that retained in the mycelium. The quantity of enzym in the mycelium was found to increase with a decrease in the temperature down to and including 9° C. Similar results were obtained with the solution, except that a slight reduction resulted in the quantity of enzym produced when the temperature was lowered from 20° to 9°.

3. The nonparasitic species (*microsporus* and *chinensis*) produced a considerable quantity of enzym, whereas *nigricans*, one of the parasitic species, produced a very small amount.

4. A comparison was made of the relative length of time required by the enzym produced by the different species to macerate the tissue of freshly dug sweet potatoes and of those which had been held in storage for several months. The fungi were grown at three different temperatures: 40°, 30°, and 9° C., maceration being carried out at 40°. In general, it was found that the middle lamellae of old potatoes were dissolved in about one half the time required to macerate the tissue of new ones.

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# THE RELATION OF SOIL MOISTURE TO THE FUSARIUM WILT OF THE TOMATO[1]

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In an earlier article (3) the author has discussed the relation of temperature to the Fusarium wilt of tomato. In correlation with the temperature studies, inquiry was made into the relation of various amounts of soil moisture to the development of the disease. The earlier article includes an account of the disease and of the characters and source of the pathogen (*Fusarium lycopersici* Sacc.), as well as of certain details as to experimental methods. These statements will apply in general to the moisture studies also, since the work along the two lines was carried on simultaneously.

## REVIEW OF LITERATURE

There are few records in the literature of plant pathology of controlled experiments dealing with the effect of soil moisture. However, a body of recorded observations offers suggestions as to moisture effects. In these it will be noted that the reference is sometimes directly to soil-moisture and at other times to soil aëration or drainage. Field observations have led to the belief that hot, dry weather favors the Fusarium wilt. Humbert (8) has recently made this statement, and, as he has supported the statement with meteorological data, there seems to be no reasonable doubt that practically every serious outbreak of the disease comes during hot, dry weather.

From a consideration of parasitic plant diseases in general, the most definite fact which has been brought out concerning soil-moisture effects has been that with saturation of the soil the host-parasite balance may be completely overturned. Recent literature indicates that usually this has resulted in a great increase in the amount of disease. Thus Johnson (9), in carefully controlled work with the Thielavia root rot of tobacco, found that saturation of the soil greatly increased the amount of disease. Rolfs (14) has reported that Rhizoctonia on a number of crops is most severe on poorly drained lands, and improvement of drainage has been recommended as a control measure for a wide variety of soil troubles. Hole (7), of the Indian Department of Agriculture, says that the sal-root fungus, *Polyporus shoraae*, is widely distributed throughout the sal forests of India, but that, so far as is known at present, it does damage only in those wet forests of Bengal and Assam in which conditions of soil aëration are known to be

[1] Investigations carried on at the University of Wisconsin under advisory relations with Professors L. R. Jones and E. J. Kraus, to both of whom the author is indebted for counsel and criticism.

particularly unfavorable. He further notes that by promoting a diseased and sickly condition in the roots, poor soil aëration may be a factor of great importance in facilitating the attacks of injurious root fungi of this class.

On the other hand, in a few instances diseases have been reported as being less severe with the soil saturated than otherwise. Appel (1) reports that alder trees in Germany suffer more severely from the parasite *Valsa oxystoma* when they are growing in meadow-land than when they are in their natural swampy habitat. Peltier (13) states that when carnation plants growing in soil inoculated with Rhizoctonia were given a heavy watering and the soil was then allowed to dry out, they were killed more rapidly than were plants growing under the same conditions, except that the soils were continually over-watered.

Concerning the effects of medium and low moistures there is very little which can be said definitely. Johnson found that whether the soil was one fourth, one half, or three fourths saturated made very little difference in the amount of Thielavia root rot which appeared.

The relation of soil moisture to the growth of autotrophic plants has been the object of considerable investigation, and in general the results indicate that there is a very wide range of moistures at which plants will grow well. Fowler and Lipman (5), reporting on work with lemon trees, state that the range of optimum or nearly optimum conditions of moisture is relatively wide. They also found that, as the moisture content was raised above the optimum, the growth curve broke sharply. As moisture decreased from the optimum, however, the rate of growth fell off very gradually. Thus the moisture curve was characterized by a gradual rise to the optimum and a sharp drop from the optimum to saturation. Kiesselbach (10) secured best growth of corn at 60-percent saturation. He noted also that the plants grew well from 20-percent to 98-percent saturation. Harris and Maughn (6), working with wheat, secured the highest yield of grain with a soil having a moisture content of 20 percent throughout the growing season, this being equal to approximately two-thirds saturation. The problem of soil moisture in relation to the growth and activity of soil fungi has received little attention. Waksman and Cook (15), and more especially Coleman (4), have published results of experiments in which they grew a variety of soil-inhabiting fungi in soil cultures of different moisture contents. The results showed that the moisture requirements of fungi differ considerably. Thus a dry medium favored maximum growth of *Aspergillus niger*, while *Trichoderma koningi* grew best on a moist medium.

## Methods of Controlling Soil Moisture

Prior to beginning these experiments, a survey was made of the literature, with the object of securing information on the technique of controlling soil moisture. In what might be called the "practical" experiments, involving economic crops, the custom has been to weigh the plants, container, and

soil at frequent intervals, sufficient water being added at these times to replenish losses. With this method the moisture is not kept absolutely constant, the range of fluctuation depending on the frequency of weighing and the rate at which water is lost. In an effort to maintain soil moisture at constant, fixed values, and at the same time to do away with some of the laborious weighing, Livingston and his associates have devised the auto-irrigator. This makes good the loss of moisture from the soil as rapidly as this loss takes place. The auto-irrigator was given a trial, but it did not function properly with low soil moistures because of poor capillarity. The capacity of the auto-irrigator, furthermore, was insufficient to supply the needs of a large, rapidly transpiring plant.

After both these methods had been used with various modifications, the following combination was finally decided upon. With dry soils, *i.e.*, those having a moisture content of 20 percent or below, the ordinary paraffin-seal method was adopted, the water being introduced through a glass tube leading from the surface to an inverted pot buried in the soil. The rate of loss of water from these low-moisture crocks of soil was slow, and the soil-moisture content was kept constant by weighing every two or three days and restoring the original weight by the addition of water. Measured amounts of water were also added between weighings. The auto-irrigators, as described by Livingston and Hawkins (12), were installed in those crocks which were to be run with a medium (23 percent) to high (35 percent) moisture content. Soils held at these moistures did not have the surface sealed, the only covering being a layer of non-absorbent cotton. The crocks were weighed every other day, and water was added to the surface of the soil when necessary. The gross weight necessary for a certain percentage of soil moisture was known, also the weights of all materials other than soil. The method of moisture control, as outlined, was fairly satisfactory from the standpoint of manipulation and possessed certain other distinct advantages. The surface of the soil with a high moisture content was not sealed with paraffin, for in an early experiment sealing was found to exert an inhibitory effect on growth if the experiment was continued for two weeks or more. This was not found to be the case, however, with very low soil moisture.

Bergman (2) has recently shown that shortage of oxygen is a factor which limits growth in the case of saturated soils. Obviously, then, if one of the deleterious effects of high soil moisture is the limitation of oxygen supply to the roots, any technique which tends still further to limit the oxygen supply will affect the results of a moisture experiment. Thus, a soil having the pore spaces filled with water until aëration had been reduced to a minimum would respond in a very marked way if the surface was sealed tightly, such treatment under these circumstances having the same effect as more moisture. Under low soil moisture, oxygen supply is not the factor limiting growth, and even with the soil surface sealed there is abundant aëration.

The surface was sealed in this case in order to prevent the formation of a hard crust as the result of excessive evaporation from the surface and a break in the capillary movement from below.

Koehler (11), working earlier in this laboratory, found in quantitative experiments that growth with low soil moisture is not affected by sealing the soil surface, but that with higher soil moisture this sealing brings about a marked inhibition of growth. He also found that with very dry soil, in containers, there were great differences in moisture content at different soil levels, unless the surface was sealed. With the higher moistures, where capillary movement was very free, this did not hold except to a very slight extent.

In the manipulation of the soil to obtain uniformity in soil-moisture content, an important factor is the degree of compactness of the soil mass. Various writers have noted the possibility of error arising from differences in this respect. The method finally adopted in these experiments, in order to obtain relative constancy, was to pack the soil firmly into the crocks and set in the plants. These crocks were then allowed to stand for a month or more, by which time the plants were well established and the soil was very compact, as would be the case in the field a month or more after planting. Then, just prior to the beginning of the actual experiment, one or more of the crocks was saturated, water being added until a slight excess of free water remained on the surface. The value secured in this way was taken as the saturation point. This saturation point varied with the height of the containing crock and with the degree of compactness of the soil. In series II, low crocks freshly filled with soil gave a saturation value of 40 percent. In series III and IV, the same soil, in tall crocks which had been allowed to stand and settle for six weeks after they had been filled, had a saturation value of 35 percent. When the soil was actually saturated, whether this required 35 or 40 percent of moisture, the development of wilt disease was inhibited, while with the soil moisture just below saturation, whether the percentage content was 32 percent or 37 percent, the wilt developed rapidly. All percentages of moisture are expressed in terms of wet weight.

## Experimental Results

*Experiments I and II.* These were in the nature of preliminary experiments and were not satisfactory in certain respects. In experiment I, conducted in the spring of 1919, the temperature was not sufficiently high to permit development of the wilt in a virulent form. The crocks were carried at low, medium, and high moisture, the actual percentages maintained being as follows:

Soil high in organic content:
  18 percent, 27 percent, 40 percent (saturation).
Soil low in organic content (a sand loam):
  11 percent, 20 percent, 30 percent (saturation).

Infection was secured at medium and low moistures, but none at saturation.

Experiment II was carried on the following fall; in this case the temperature conditions maintained were favorable for a maximum development of the disease. The regulation of moisture was not entirely satisfactory and the methods of planting and handling the crocks were not yet perfected. The results of this experiment, however, showed decisively that plants grown in saturated soils do not develop the wilt. With high moisture content which did not quite reach saturation (30–35 percent), the wilt developed, and the same was true with medium moisture content (25–30 percent). There was a distinct falling off in the amount of disease in soils which were dry enough (22 percent and below) markedly to check growth. The results from this series are summarized in table 1.

TABLE 1

| Soil Moisture, Percent | No. Plants | Percent Healthy | Percent Diseased | Percent Dead |
|---|---|---|---|---|
| 18–25 | 19 | 47 | 31 | 22 |
| 26–27 | 27 | 15 | 59 | 26 |
| 40 (saturation) | 12 | 100 | 00 | 00 |

*Experiment III.* This experiment was begun December 22, 1919, and completed March 1, 1920. The soil was steam-sterilized and inoculated with a spore suspension of *Fusarium lycopersici*. One-gallon crocks were filled, planted, and carried into a cool temperature (15°–20° C.) greenhouse. Here the plants grew for a period of four weeks, with an air temperature of 15° to 20° C. and a soil temperature averaging about one degree less. There was no development of the disease at these temperatures.

On January 19, 1920, a series of crocks containing inoculated plants was carried into a temperature favoring the disease (about 28° C.), the soil having previously been adjusted to various moisture contents.

On February 20 the condition of the plants was as shown in table 2.

TABLE 2

| Moisture, Percent | No. Plants | No. Diseased | No. Dead |
|---|---|---|---|
| 14–16 | 4 | 0 | 0 |
| 16–18 | 4 | 3 | 1 |
| 23–24 | 4 | 4 | 3 |
| 26–28 | 4 | 3 | 2 |
| 31–33 | 4 | 4 | 4 |
| 35 (saturation) | 2 | 0 | 0 |

On March 1 the experiment was discontinued and the data taken are shown in table 3.

*Experiment IV.* The tomatoes used were planted December 22, 1919, and grown at the low temperature (15°–20° C.) until February 9, 1920.

At this latter date they were transferred to the warm-temperature house, the soil having first been brought to the moisture content desired for the experiment.

TABLE 3

| Moisture, Percent | No. Plants | No. Diseased | No. Dead |
|---|---|---|---|
| 14–16 | 4 | 1 | 0 |
| 16–18 | 4 | 4 | 1 |
| 23–24 | 4 | 4 | 3 |
| 26–28 | 4 | 4 | 3 |
| 31–33 | 4 | 4 | 4 |
| 35 (saturation) | 2 | 0 | 0 |

On March 15 the experiment was concluded, and the data taken are shown in table 4.

TABLE 4

| Moisture, Percent | No. Plants | No. Diseased | No. Dead |
|---|---|---|---|
| 13–14 | 4 | 0 | 0 |
| 15–16 | 4 | 1 | 1 |
| 17–18 | 2 | 2 | 0 |
| 22–24 | 4 | 4 | 3 |
| 26–28 | 2 | 2 | 1 |
| 28–32 | 3 | 3 | 3 |
| 35 (saturation) | 2 | 0 | 0 |

These experiments showed that the plants growing in the driest possible soil were highly resistant to the wilt disease in spite of the fact that these plants wilted badly during the middle of the day from lack of water. The most rapidly growing plants were attacked and killed first, while plants growing in saturated soil seemed immune since they were never attacked by the disease. During the progress of these experiments data were taken on the incubation period of the disease under the different conditions of soil moisture. The data given in table 5 are from experiment IV.

TABLE 5.

| Moisture, Percent | No. Days Prior to First Appearance of Disease | Incubation Period (Average) |
|---|---|---|
| 13–14 | 35 | 35 |
| 15–16 | 24 | 24 |
| 17–18 | 26 | 26 |
| 22–24 | 15 | 19 |
| 26–28 | 18 | 20.5 |
| 28–32 | 15 | 19 |
| 35 (saturation) | .. | — |

These data again bring out the point that the disease makes its most virulent development with medium and high soil moisture. The plants growing in dry soil were slow to produce visible signs of the disease, and the development of the trouble, even after it had affected the lower leaves, was very much retarded.

*Experiment V.* In the work up to this point the principal purpose was to determine the effect of constant differences in soil moisture, the moisture content ranging from very nearly the minimum for life to complete saturation. The problem of sudden shortage of moisture in connection with a rise in temperature which favored the disease was not directly attacked. However, as has been brought out, the plants growing in soil so dry that the foliage was in a semi-wilted condition most of the time were highly resistant to the wilt disease, while, on the other hand, the plants growing under the conditions of soil moisture most favorable to growth were the first to be attacked.

On January 15, 1920, fourteen one-gallon crocks were filled with sterilized soil which had been inoculated with a spore suspension of *Fusarium lycopersici*. The weights of the crocks, soil, clay cups, etc., were recorded, and two plants were set in each crock. These crocks were then kept in the cool greenhouse until March 13, at which time the first blossom clusters were well developed. At the end of this period the plants were moved into a compartment where the temperature (about 28° C.) was favorable to the disease, and there subjected to the following moisture conditions:

Two crocks were saturated first, the remaining twelve being divided into four lots of three each. The experiment was continued for a period of 5 weeks, as follows:

Lot A: The soil was kept moist for all 5 weeks.
Lot B: The soil was kept very dry for the first week and moist for the next 4 weeks.
Lot C: The soil was kept moist for the first week and dry for the 4 weeks following.
Lot D: The soil was kept very dry for all 5 weeks.

The plants of lot A made a very luxuriant vegetative growth; those of lot B were noticeably affected by the week of drought, but grew rapidly after this. Those of lot C grew very rapidly during the initial moist period, and suffered extremely when the soil was suddenly allowed to become dry. Many of the tender tips of the leaves dried up, and the stems became hollow. The plants of lot D were dwarfed and woody, and were practically the same size at the end of the experiment as they were at the beginning.

The data with regard to disease are summarized in table 6.

TABLE 6

| Lot | Treatment: Weeks Dry | Treatment: Weeks Moist | Length of Incubation Period in Days | No. Plants Dead or Diseased after 5 Weeks | No. Appearing Healthy |
|---|---|---|---|---|---|
| A.... | 0 | 5 | 15 | 6 | 0 |
| B.... | 1 | 4 | 22 | 6 | 0 |
| C.... | 4 | 1 | 24 | 1 | 5 |
| D.... | 5 | 0 | Experiment discontinued at the end of 35 days | 0 | 6 |

The results of this experiment were in line with those secured previously, and, when considered in connection with the preceding work, make possible the following conclusions:

1. Plants growing very rapidly as a result of optimum moisture conditions for vegetative growth are most susceptible to the wilt.

2. Moisture shortage that checks the growth of the host plant checks the development of the wilt also, and the longer and more severe the period of drought, the more the disease is inhibited.

3. Plants growing in saturated soil are never attacked by the wilt disease.

## The Relation of Soil Moisture to the Growth of Uninoculated Plants

Check series, consisting of plants grown in inoculated soil, were run with experiments I, II, and III. The data from check series III only will be considered here, as they are the fullest. This series consisted of single crocks, each containing two plants which were carried at the different soil moistures for a period of seven weeks with an air temperature of approximately 28° C.

The data given in tables 7 and 8 were taken at the conclusion of the experiment.

Table 7

| Soil Moisture, Percent | Weight of Tops Produced | | |
|---|---|---|---|
| | Wet Weight, Grams | Dry Weight, Grams | Percentage Dry Weight |
| 14–16 | 14.7 | 2.4 | 16.3 |
| 16–18 | 23.5 | 3.1 | 13.1 |
| 19–21 | 75 | 9.6 | 12.8 |
| 23–25 | 102 | 11.37 | 11.1 |
| 31–33 | 146 | 15.6 | 10.6 |
| 35 (saturation) | 15.7 | 2 | 12.1 |

Table 8

| Soil Moisture, Percent | Microchemical Analysis of Stem | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tip of Plant | | | Base of Plant | | | Tap Root | | |
| | Nitrate | Sugar | Starch | Nitrate | Sugar | Starch | Nitrate | Sugar | Starch |
| 14–16 | 2 | 2 | 2 | 2.0 | 2 | 2 | 2 | 2 | 1 |
| 16–18 | 2 | 3 | 2 | 2.5 | 2 | 2 | 2 | 2 | 1 |
| 19–21 | 2.5 | 5 | 2 | 3 | 5 | 2 | 2.5 | 2 | 1 |
| 23–25 | 3 | 5 | 2 | 5 | 5 | 1.5 | 2.5 | 5 | 1 |
| 31–33 | 2 | 5 | 2 | 3 | 3 | 2 | 2.5 | 2 | 1 |
| 35 (saturation) | 1 | 3 | 1.5 | 1 | 5 | 1.5 | 1.5 | 2 | 1 |

1, absent. 2, slight. 3, moderate. 4, abundant. 5, very abundant.

From these data it can be seen that the disease-resistant low-moisture

plants were high in dry weight and low in nitrates and sugars. The susceptible plants, grown under good moisture conditions, were rich in nitrates and carbohydrates but low in dry weight. The plants growing in saturated soil, which were immune to the disease, showed one striking difference from all others—they were almost destitute of nitrates.

It may be noted that this series was grown during the darkest portion of the year, January and February. In experiment III, which was conducted at the same time, the blossum buds were all abscised as soon as the plants were moved from the cool temperature (15°–20° C.) to the warm temperature (25°–30° C.). In experiment IV, carried on during February and March, there was abundant flowering at the medium and high soil moistures after the plants had been moved into the warm temperature. Since the moisture and temperature conditions were practically the same during the two experiments, this difference in flowering may probably be attributed to light conditions. In this locality (Madison, Wisconsin) the light is very much reduced for several months prior to the middle of February. After that time, however, there is a rapid increase in the amount of sunlight. This increase in sunlight facilitated carbohydrate manufacture and changed the carbohydrate-nitrogen ratio, with the result that the plants changed from the vegetative to the reproductive condition. This difference, however, was not correlated with a difference in the resistance or susceptibility of the plants to wilt, the behavior of the disease being the same in experiments III and IV.

## Experiments Dealing with the Immunity Induced by Saturation of the Soil

After it had been definitely shown in experiment II that the plants growing in saturated soil were not subject to the wilt, the reason for this relative immunity was investigated. It was soon found that, if these plants in saturated soil were allowed to grow under conditions of medium soil moisture (25–30 percent) for a short time, their immunity to disease was lost. Thus, after the plants had resumed the "normal" type of growth, they were attacked by the disease and killed. It was further found that the period of time between removal of the plants from the saturated soil condition and the date of first appearance of the wilt was the same as the incubation period of freshly inoculated plants. This fact led to the conclusion that in the case of the plants in saturated soil the fungus was able to make little or no progress up the stem of the host as long as the soil remained saturated.

To corroborate these conclusions as to the inability of the parasite to grow in the tissues of the plants grown in saturated soil, these plants were taken out and dissected at different times. In several instances slightly discolored bundles were found in the base of the stem, but in no case was it possible to plate out the fungus from the stem. The lower roots of these

plants were found to be much decayed, only a few roots thrown out at the very surface of the soil being white and clean in appearance. Portions of partly decayed roots were plated out, and cultures of saprophytic fungi and bacteria were secured. In one instance, however, a root was plated out which gave pure cultures of *F. lycopersici*, the fungus apparently growing out from the decayed root cortex only. These cultures were preserved and proved through inoculation experiments to be virulently pathogenic.

These and other considerations have led to the view that the immunity induced by soil saturation is probably a host-plant factor. Thus the fungus grows very well in liquid culture media, and it may be present in the partially rotted roots. Furthermore, these plants throw out many new roots at the surface of the soil; through these roots infection could readily take place if infection were possible.

From microchemical analysis it was found that the saturation plants were markedly different from normal plants in their nitrogen relations. Resistance in these plants seemed to be correlated with the absence of nitrate nitrogen. This relationship was tested by growing plants in sand culture and adding nutrient solutions. A complete nutrient solution was supplied to part of the plants, and a solution lacking nitrate to the remainder. The temperature conditions in the greenhouse where this work was conducted did not permit a virulent development of the disease, but it was conclusively shown that plants grown without nitrate, the tissues of which plants give no nitrate test, are not infected by the fungus. Plants grown with a complete nutrient solution were readily infected.

## Experiments Dealing with Resistance Induced by Low Soil Moisture

It has been shown by carefully controlled experiments that succulent, rapidly growing plants produced under optimum moisture conditions (23–33 percent) are subject to the disease, while the woody, slowly growing plants which result from low moisture (13–20 percent) are not readily attacked.

Rapidly growing, susceptible plants were made resistant by allowing the soil to dry, the growth being checked in this way. Slowly growing, resistant plants were made susceptible by making the soil moist, and thus inducing a rapid, succulent growth of the host. Thus, at the conclusion of experiment V there were set aside eleven plants which had been growing with the soil very dry and at a temperature of about 27° C. for five weeks without showing the wilt. The soil of four had been made moist; the remainder were allowed to continue dry. In a week the four plants in the moist soil had changed over into a succulent, rapidly growing condition, and in three days more all were showing the initial symptoms of wilt. The disease progressed rapidly, and soon the plants were completely wilted. The seven plants which had been allowed to remain dry during this time did not develop any disease and were in the same condition at the end of the experiment that they had been in at the beginning.

Thus the disease could be brought on by making the plants from the saturated soil (35 percent moisture) dry, or the plants from the dry soil (13–20 percent) moist. The reduction in amount of disease with dry soil and saturated soil appears in each case to be due to, or at least correlated with, detectable differences in the host plant. The resemblance between the two ceases, however, at this point.

When grown at a temperature favorable for the disease, and with dry soil, plants are infected and the fungus progresses slowly up the stem. If the temperature conditions are optimum for a sufficiently long period, these plants are killed by the wilt. In experiments IV and V the shortest incubation period for plants growing with 23 to 33 percent of soil moisture was, in each case, 15 days; the shortest incubation period for plants grown in the driest soil was 35 days. In numerous instances, apparently healthy plants growing with 13 to 14 percent soil moisture have been cut and found to have vascular discoloration extending far up the stem. The fungus was readily isolated from such plants, and in addition it was isolated in a number of instances from the tissues of plants which showed no browning of the bundles. These investigations have indicated that a very large majority of the low-moisture plants which do not show external symptoms of the wilt are nevertheless infected.

The presence of such infection can be readily shown in another manner. The temperature conditions were such that, with a soil moisture of 25 to 30 percent, the disease made its appearance in about 15 days. At the conclusion of experiment V, as already noted, four dry-soil plants which showed no disease were made moist; all four showed the disease in 10 days. Since it had been definitely proven that under the given conditions 15 days was the least possible time in which the fungus could infect, grow up the stem, and produce visible wilt, the only explanation for this result in 10 days is that infection had already occurred during the long period of weeks when the plants were growing in very dry soil, but at a temperature optimum for the disease.

Since the dry-soil plants are readily infected, and the fungus, once within the host, produces the disease very slowly, it seems reasonable to suppose that the host as a whole is resistant and that this fact is responsible for the great reduction in the amount of disease.

In the plants grown in saturated soil, infection did not occur, and the incubation period was not reduced by exposing the plants to a soil temperature between 25° and 30° C., for a long period with the soil saturated. There was no evidence that the disease would ever develop, or infection occur, so long as the saturated condition was maintained. For these reasons the dry-soil plants are characterized as resistant, while the saturated-soil plants are said to be immune.

## Comparison of the Effects of Low Soil Moisture and Low Soil Temperature on the Host, in Relation to Development of the Disease

The resistance induced by low soil moisture seems different from that induced by low soil temperatures. Thus, plants could be produced which had tops that were susceptible and roots which were resistant to the disease, or roots that were readily invaded and tops that were not attacked. A soil temperature of 15° to 20° C. checked the progress of the fungus while it was in the roots, but exerted no influence if the parts above ground became infected, the temperature of the air being the deciding factor in that case. On the other hand, soil-moisture conditions low enough (14–18 percent) to check the disease exert an influence through the plant as a whole, roots and tops both being made resistant.

Temperature effects on disease were not correlated with apparent differences in the host plant; thus, the decrease from a minimum of disease at a temperature of 27° to 30° C. to no disease at 19° to 20° C. was not associated with a marked change in the appearance or in the composition of the host plants.

The case of soil moisture was different, for the decrease from a maximum of disease with a soil moisture of 30 to 33 percent to a minimum of disease with a soil moisture of 13 to 14 percent was correlated with a marked reduction in the vegetative vigor of the host plant. This decrease in the amount of disease did not begin to manifest itself until the soil moisture was reduced to a point where the plants actually began to show symptoms of moisture shortage, and the more acute this shortage became, the more pronounced was the decrease in disease. The plants grew well with soil moistures from 22 to 33 percent, and there was a virulent development of the disease. The plants growing with a soil moisture of 18 to 19 percent were the first to show perceptibly increased resistance to the disease, and likewise the first to show a marked reduction in vegetative vigor of growth. During periods of excessive transpiration they suffered severely. A soil moisture of 13 to 14 percent brought the plants close to the point of permanent wilting, and these plants showed a maximum resistance to disease. Thus the evidence now at hand indicates that soil moisture and soil temperature act in different ways to increase or diminish the amount of disease.

## Interpretation of the Temperature and Moisture Results in Terms of Field Conditions

In the early work with temperature no attempt was made to control soil moisture accurately, water simply being added every day in amounts sufficient to keep the soil moist. In the later experiments the moisture was held constant by weighing the pots and adding the amount of water needed to restore them to the constant weight. However, since the range of moisture conditions almost equally favorable to the development of the disease

is very wide, 22 to 33 percent with the soil used in the moisture work, there was little object in controlling moisture exactly in the temperature experiments.

Early in the work, owing to the fact that the disease appeared to develop equally well over a wide range of soil moistures, it was thought that soil moisture was of very minor importance as compared with temperature. However, it now seems that extreme drought would certainly check the development of the disease, regardless of temperature conditions.

A brief consideration of the application of these findings to field conditions follows. The moisture results indicate that for a virulent development of the disease the plants must be rapidly growing. The temperature results showed that warm air and soil temperatures (27°–31° C.) were essential for a virulent development of the disease. Therefore it would be expected that, under field conditions, a rainy period, inducing rapid growth, followed by hot weather, would furnish optimum conditions for the quick appearance and the rapid progress of the disease. Weather which was hot and moist would be favorable, but it was pointed out in the temperature work that an even warm or hot temperature is not nearly so effective as the same temperature with intermittent periods of extreme heat. Bright, sunny days furnish this intermittent temperature, while during hot, moist weather the temperature is rather even. A warm, rainy period followed by hot weather would produce the disease more quickly than cool, rainy weather followed by a hot period, since under the former conditions infection would have occurred and growth of the fungus up the stem would have begun. Continuously dry weather which checks the growth of the plants should also check the development of the disease.

## Summary

Tomato plants were grown in crocks of sterilized soil inoculated with a spore suspension of *Fusarium lycopersici*. The soil in these crocks was held at moisture contents ranging from 13 to 35 percent, the higher value representing complete saturation.

The plants growing in soil with a low moisture content, 13 to 19 percent, were very resistant to the disease.

The plants growing in soil which was kept saturated were immune from attack.

In general, it was found that any moisture shortage sufficiently severe to check the vegetative vigor of the host checked the disease proportionally.

When rapidly growing plants held at a temperature below 20° C. were brought into a temperature favoring the disease (25°–30° C.), they were soon attacked by the wilt. However, if the soil was allowed to dry out as soon as the plants were placed at the warm temperature, the appearance of the wilt was very much delayed. Thus, rapidly growing succulent plants, which had been susceptible to the disease, were made disease-resistant by allowing the soil to become very dry.

Plants growing in soil with a very low moisture content lost their disease resistance if a rapid, vegetative type of growth was induced by the addition of sufficient water to keep the soil moist.

Plants growing in saturated soil were immune to attack, but if the moisture content was lowered the disease soon developed.

The immunity of the saturated soil plants was apparently correlated with the practical absence of nitrates in the host tissues.

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## EXPLANATION OF PLATES

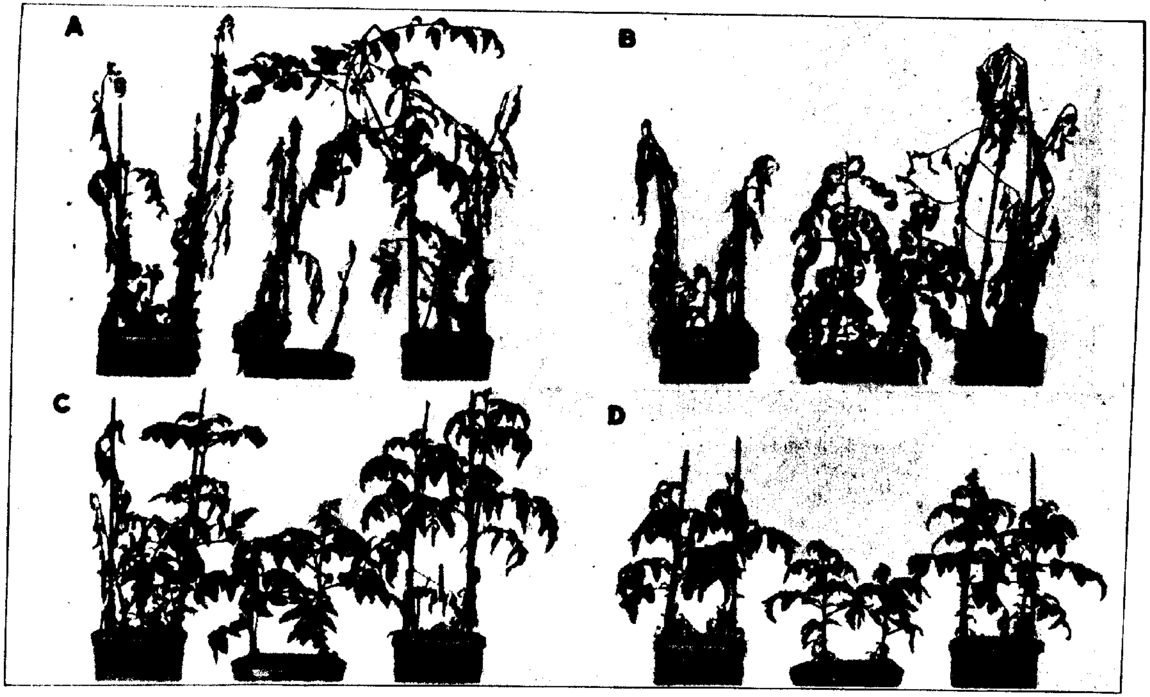
### Plate XIII

*A*. A series of plants growing in sterilized, uninoculated soil with soil moisture ranging from 15.5 to 35 percent (saturation). At 32 percent, the maximum wet weight and the maximum dry weight of tissue were secured; the percentage dry weight, however, was the lowest at this percentage of moisture. The plants growing with 15.5 percent moisture were low in total weight but high in percentage dry weight. The plants growing in saturated soil (35 percent soil moisture) were low both in total weight and in percentage dry weight.

*B*. Plants grown in soils inoculated with *Fusarium lycopersici* at the same time as the plants of the check series (*A*), the soil moistures used in the two series being the same.

CLAYTON: SOIL MOISTURE AND FUSARIUM WILT


CLAYTON: SOIL MOISTURE AND FUSARIUM WILT




CLAYTON: SOIL MOISTURE AND FUSARIUM WILT

It will be observed that the disease affected the plants growing in soils with moistures from 20 to 32 percent. One of the plants in the 20-percent crock was still alive at the time the photograph was taken, while both plants in the 23.5- and 32-percent crocks had been dead and dry for some days.

PLATE XIV

*A*. These plants had been growing with moistures of 14, 18, 25, 30, and 35 percent (saturation) for two weeks. The soil was inoculated with *Fusarium lycopersici* in pure culture. Infection showed in the 25- and 30-percent crocks the day after the photograph was taken, and within two weeks these plants, which were making the most rapid growth, were dead. The temperature ranged from 25° to 30° C.

*B*. Four of the five crocks of plants shown in figure *A*, the 18-percent soil-moisture crock being omitted because of lack of space. This photograph, taken two weeks after the one above, shows how the wilt disease attacks and destroys tomato plants growing under good conditions, while plants growing in very dry soil (14 percent) or saturated (35 percent) were not attacked. The semi-wilted condition of the plants growing in the crock of soil held at 14 percent soil moisture is due to moisture shortage, not to attack by the disease.

PLATE XV

The plants in lots *A*, *B*, *C*, and *D* were grown five weeks in sterilized and inoculated soil, with temperature conditions favorable for the development of the disease.

The following sets of soil-moisture conditions were maintained:

Lot *A*: 5 weeks with moist soil.
Lot *B*: 1 week with dry soil, 4 weeks with moist soil.
Lot *C*: 4 weeks with dry soil, 1 week with moist soil.
Lot *D*: 5 weeks with dry soil.

The photographs were taken at the end of the five-week period. The disease developed with maximum virulence in lot *A*; in lot *B*, the plants were almost as severely affected; lot *C* had only one plant affected by the wilt; lot *D* showed no disease. The development of the disease was thus directly in proportion to the amount of moisture supplied. It is interesting to note that in lot *B*, one week of dry soil conditions having preceded four weeks of moist soil conditions, the incubation period for the disease was 22 days, while in lot *A*, good moisture conditions having been maintained for five weeks, the incubation period was 15 days.

# STUDIES IN THE MORPHOLOGY OF *RICCARDIA PINGUIS*

Amos M. Showalter

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*Riccardia pinguis* (L.) S. F. Gray is found rather sparingly along the edge of the marsh next the wooded upland south of Lake Wingra (Madison, Wisconsin). The thalli grow on fallen willow canes, on the bases of standing willows, on the black mud, and occasionally on fallen leaves. In this locality they are well shaded during the summer when the willows and oaks are in full foliage, but are partly exposed during the fall and early spring.

Plants of this species are somewhat more abundant on the railroad right-of-way through the swamp prairie bordering Lake Waubesa and extending westward for about a mile. At this station the thalli grow on the mud and cinders and more rarely on the roots and stubble of grasses. The grasses stand one half to one and one half meters high and form a fairly thick shade over the thalli during the summer months, but are burned off annually in the fall or spring. Numerous searches in the swamp on either side of the railroad have revealed no plants beyond the limits of the annual burning of the grass.

In June, 1921, before the latter station had been discovered, the low prairie in the vicinity of Roby, Indiana, about twenty miles from Chicago, was visited in the hope of obtaining material for cytological study. Plants were found in this region growing chiefly on decaying leaves about the bases of very small willows scattered through the more swampy portions of the prairie. This material was not in thriving condition because of insufficient moisture, and only a small collection was made.

At all these stations the plants are protected from the summer sun but are favored with nearly full sunlight during the fall and early spring—the seasons during which vegetative growth appears to be most rapid. It was further observed that plants exposed, by tramping of the grass, to the August sun quickly succumbed.

## Field and Culture Observations

The first collections of plants for this study were made in the spring of 1920 from the region of Lake Wingra and consisted of only a few dozen thalli. These plants were used to start greenhouse cultures which grew very well during the summer and early fall of 1920, but later became contaminated with blue-green algae and had to be discarded early in the following winter. These cultures were grown on a thin layer of leaf mold over a substratum of sand in shallow wooden boxes standing in a tray of nutrient

solution (formula of Moore, 1903). One of these cultures, started with about a dozen small thalli identified as female, spread over and completely covered an area of about one and one half square decimeters. It produced hundreds of archegonia but bore no sporophytes, there being no male plants in the culture. Another culture, male and female mixed, in a box four decimeters by three decimeters in area, completely covered the ground, mostly with several layers, and spread over the edges of the box. This culture was cared for by Dr. W. N. Steil during my absence of twelve weeks in the summer of 1920, and upon my return in September it was noticed that the male plants were being covered by the overgrowing female plants, in which young sporophytes were appearing rapidly. A few weeks later the culture appeared to be all female with developing sporophytes in large numbers. Most of the sporogonia when nearing maturity were eaten by insects, and the thalli succumbed to algal overgrowth.

Living plants bearing mature sporophytes were received February 11, 1921, from Mr. Severin Rapp, of Sanford, Fla. These, with the decayed wood on which they had grown, were placed on leaf mold underlain by sand in shallow earthen pots and plates and were saturated thoroughly with nutrient solution. On the day following they appeared to have recovered from the effects of shipping, and about two hundred of the youngest sporogonia were fixed in Flemming's solutions: the medium, the strong, and the strong diluted with an equal volume of distilled water. On sectioning these later, a few were found to show stages of the heterotypic and homoeotypic divisions; in others these divisions had been completed, and in some they had not yet begun.

A few of the sporogonia had already opened when they were received, and most of the remaining three or four hundred (estimated) discharged their spores within three weeks. Miss Clapp (1912) states that the spores when discharged often adhere in tetrads, but I have been unable to confirm this observation. The discharge of about a dozen capsules was observed under the binocular dissecting microscope; the spores appeared perfectly spherical except for the echinate surface and none could be found adhering in tetrads. However, it has more recently been found possible to obtain viable spores in tetrads by soaking the ripe capsules for several hours in water and then opening them under water.

Culture experiments with this plant have generally been unsatisfactory, and I have not yet succeeded in growing mature thalli from spores.

Two of these cultures of Florida material are still in thriving condition after sixteen months, although the plants have not grown very extensively. One culture, in a pot nine centimeters deep by eleven centimeters in diameter, appears to be almost entirely female, but has produced relatively few sex organs. The other culture, in an earthen saucer five centimeters deep by twenty-eight centimeters in diameter, is mixed, some parts being predominantly male and other parts mostly female. The plants in this

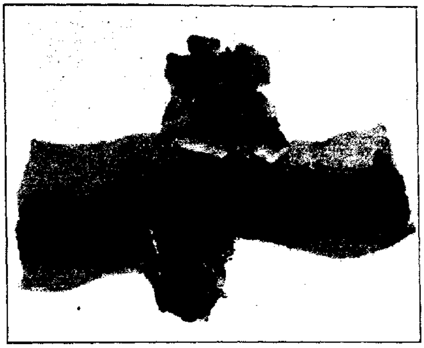
culture began producing sex organs early in the spring (1921) and produced great numbers of antheridia and archegonia until December, except during the hot summer months when the plants grew but little and appeared about to succumb. Numerous attempts to obtain functional antherozoids resulted only in the discharge of imperfectly formed, non-motile, coiled bodies. A few female plants with archegonia were fixed late in December and showed disintegration of the axial row before the maturing of the archegonium. No sporophytes have appeared in these cultures.

The form of the antheridial branches in these cultures was noticeably different from that of the antheridial branches of plants collected in the vicinity of Madison. These branches in the Florida material grown in the greenhouse were borne in groups of three (rarely five) as figured by MacVicar (1912, p. 50). The individual branches were short and wide, with thin upturned or inrolled margins. The antheridia were arranged rather irregularly. Representative plants were sent early in the spring (1921) to Professor A. W. Evans. He wrote that "the male branches are certainly very peculiar and I have never seen anything quite so indefinite among the various specimens of Riccardia which I have studied." He also commented upon the wide distribution of this species and suggested that "under the circumstances a considerable range of variation is to be expected."

The antheridial branches produced in these cultures from early spring to December showed no significant variations in form; but a few male plants sent by Mr. Rapp from the same locality in Florida and received October 8, 1921, bore antheridial branches so different from those of the Florida plants grown in the greenhouse that they might easily have been taken to be of a different species. The plants in this latter shipment bore antheridial branches ranging up to four or five millimeters in length, very uniform in width from base to tip, and with two (occasionally four) alternating rows of pits from which the antheridia had disappeared and which were arranged with almost mathematical regularity. Miss Clapp (1912), working on material collected at Xalapa, Mexico, described the arrangement of the antheridia on the branches as "extremely regular, in two alternating rows corresponding to the segments of the apical cell." The appearance of the plants in this latter shipment from Florida is certainly in accord with her description. In those branches having four rows of pits it seems evident that each branch had possessed two apical cells, the segments from each apical cell having given rise to two rows of antheridia. In the plants collected in the vicinity of Madison, the antheridial branches are generally borne singly, are relatively short and wide, and show little regularity in the arrangement of the antheridia (text fig. 1). It seems probable that the form of the sexual branches as well as that of the vegetative shoots in this species is readily affected by environmental conditions.

The most fruitful source of material (that near Lake Waubesa) was discovered August 11, 1921, when the plants bore mature sex organs in

great numbers and young sporophytes were fairly numerous. On this and the following day ten lots of material were fixed in the field, using Flemming's fixing solutions: the medium unmodified, and the strong diluted with an equal volume of distilled water. These gave equally good results; there was a slight shrinkage of the cells of the surface layer of the thallus and of the



TEXT FIG. 1. Photomicrograph of a portion of a male thallus bearing two antheridial branches, unstained, mounted *in toto* in balsam. × 12½.

cells of the archegonia, and in most cases considerable shrinkage of the cells of the young embryos. Thalli on which sporophytes were observed to be developing were generally left undisturbed in the hope that material might later be available for a study of the reduction divisions. After washing and dehydrating to eighty percent alcohol, the plants were picked over carefully under the binocular microscope and the male and female plants were separated.

The male plants in these collections averaged about one half to two thirds the size of the female plants (this is true also of a small collection of male and female plants, which had grown intermingled, collected one week earlier in the region of Lake Wingra). Two weeks later, when the Waubesa region was again visited, very few antheridia were to be seen, and the male plants were growing vigorously. Subsequent observations, when the male and female plants could be distinguished only by the vestiges of sexual branches or by the presence of sporophytes, have convinced me that there is no pronounced difference between male and female plants as to size or luxuriance of growth except during the period of gamete production, and I am not sure that the difference during this period is always so distinct as it appeared to be in the collections just described. Attempts to test this point by extended cultural experiments have thus far failed.

The plants fixed in the field August 11 and 12, 1921, near Lake Waubesa form the basis of the studies reported in this paper. This material was supplemented by greenhouse plants of both Wisconsin and Florida stock, but, except as otherwise stated, all figures were drawn from the material of these collections. The plants were imbedded in paraffin, cut into sections 10 $\mu$ thick, and stained either with Flemming's triple stain, Heidenhain's iron-alum haematoxylin, or with safranin and light green. For the observations recorded in this paper, the last-named combination proved most satisfactory.

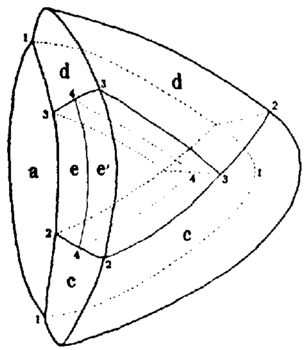
## The Apical Cell and Segmentation

Miss Clapp's (1912) description of the position, form, and segmentation of the apical cell is in harmony with the earlier accounts of Kny (1863) and Leitgeb (1877). The apical cell is a large, wedge-shaped structure with right and left cutting faces from which segments are cut off alternately (Clapp, 1912). These two cutting faces are convex and meet above, below and behind, thus constituting all the surface in contact with other cells of the thallus. The free (anterior) surface of the apical cell is relatively small and approximates an ellipse of which the longer axis is the one perpendicular to the surface of the soil or other substratum on which the thallus is growing (fig. 4, Pl. XVI). Transverse sections through the apical cell show this same elliptical outline. Figures 4–8 show serial sections of the same apical cell (*a*) sectioned transversely, each section being 10 $\mu$ in thickness. This cell tapers abruptly to a blunt point in the next serial section, not shown in the figures. (Compare also the horizontal section shown in figure 1, Plate XVI, and Miss Clapp's figures 5, 6, and 11.) The relative lengths of the different axes of the apical cell vary considerably, but the horizontal transverse (right-left) axis is always much the shortest of the three principal axes (figs. 1, 4–8; Miss Clapp's figs. 5, 6, 11). In the vegetative branch the vertical and horizontal axes of the apical cell are approximately equal (figs. 3, 10). The apical cell *a* shown in figures 4–8 appears in six vertical transverse sections, each 10 $\mu$ in thickness, thus having an antero-posterior length of about fifty microns, approximately equal to the length of its median vertical axis (fig. 6).

The division of the apical cell is vertical and almost longitudinal (figs. 1, 11), cutting off a segment which at first approximates the shape of the apical cell (figs. 1, 9–11). Such segments are formed alternately from the right and left cutting faces of the apical cell, as all previous workers agree (see especially Kny, 1863, Pl. VI, figs. 4, 5; Leitgeb, 1877, Pl. I, fig. 1). The segment appears to exceed the apical cell in size, and the side of the segment adjacent to the apical cell soon becomes markedly concave, as the apical cell resumes its biconvex shape.

Leitgeb (1877), Campbell (1905), and Miss Clapp (1912) differ in their accounts of the division of the primary segment. Leitgeb (p. 41) says that the primary segment divides like a two-faced apical cell, cutting off segments

not to the right and left, but below and above. These divisions are best represented by a diagram showing the apical cell, the primary segment, and the cells into which the primary segment is divided (text fig. 2). In this diagram the plane 1–1 ·· 1 represents the division which cuts off the




TEXT FIG. 2. Diagram of the apical cell (*a*), and of the cells into which the primary segment divides; explanation in text.

primary segment from the apical cell *a*. The first division (2–2 ·· 2) of the primary segment cuts off a ventral cell *c* (see also figs. 9, 13, 2, 4, Pl. XVI). The next division (3–3 ·· 3) cuts off a dorsal cell *d*, and intersects the preceding division wall (see also figs. 2, 4, Pl. XVI). The third division (4–4 ·· 4) is vertical, at right angles to the former two, and divides the middle cell into right and left halves, *e* and *e'* (cf. fig. 4, Pl. XVI). Apparently one of these last two cells may continue to divide as an apical cell, giving off right and left segments and becoming the axis of a new branch; or both cells *e* and *e'* may function as ordinary surface cells. I have not followed these later divisions critically, but all my observations are in perfect agreement with those of Leitgeb. Figure 9 shows a vertical longitudinal section of the primary segment (*b*) in process of its first division. This figure was drawn from the serial section adjacent to and preceding the one shown in figure 10. Figure 13 shows the secondary segments (*b'* and *c*) in an archegonial branch, and was drawn from the serial section adjacent to and following the section shown in figure 12. Figure 2, drawn from the section adjacent to and following the one shown in figure 3, shows the secondary segments (*b'* and *c*) in preparation for the next succeeding division.

Miss Clapp reports that "the primary segment is divided by a vertical transverse wall into an inner posterior cell and an outer anterior marginal one." I have observed this first division of the primary segment in a half dozen or more clear cases. All of these show this division wall in a diagonal plane intermediate between the horizontal plane and the vertical transverse

plane of the thallus, intersecting the outer exposed wall of the cell and sloping upward interiorly (figs. 2, 9, 13). In some of the cases observed the plane of division is more nearly horizontal than indicated in figures 2 and 9. It is clear that, if seen in horizontal sections, the secondary segments shown in figure 2 and those in process of formation in figure 9 would appear to be "inner posterior" and "outer anterior" cells; this is the case in the horizontal section shown in figure 1, in which *c* and *b′* are the daughter cells in question. Miss Clapp figures only the horizontal sections (see her figures 3–10), and I am unable to determine with certainty from these whether in her material the division of the primary segment is a vertical transverse one, or whether, as I have found in harmony with Leitgeb, it is really in a diagonal plane.

Campbell says that "the segment first divides into an inner and an outer cell, and the former probably next into a dorsal and a ventral one." He does not figure these divisions, and it is not clear to me whether he means that the first division of the segment is into an inner posterior and an outer anterior cell, or into an inner cell proximal to the apical cell, and an outer cell distal to it. His description of the origin of the sex organs seems to imply that he means the latter, but it is difficult to reconcile his statement with the observations of Leitgeb, or with those recorded in the present paper.

As described by Miss Clapp, the apical cell is located in a sinus resulting from the forward growth of the thallus to the right and left of this cell. Each sinus contains usually two or more apical cells, which gradually diverge as new segments are cut off and undergo further division. As the two apical cells thus diverge, the group of cells between them grows forward and divides the sinus into two; but in the meantime each apical cell usually has given rise to another, so that each new sinus contains two apical cells. The time of the formation of new apical cells seems to vary, however, so that a sinus may contain more than two or (rarely) only one apical cell. The branching is truly dichotomous, but usually one branch of each pair develops only slightly and thus becomes apparently lateral (in this connection see also Clapp, 1912, and Campbell, 1905). During the season of gamete production these lateral branches begin very early to produce sexual organs, thus becoming gametophores. Text figure 3 shows a series of transverse sections, 10 $\mu$ in thickness, of the growing end of a thallus having four apical cells (*a*) in one sinus. The portion of the thallus in the middle of the sinus had just begun to push forward in advance of the apical cells, and two young archegonia (♀) shown in photographs *A* and *B* indicate that the two apical cells uppermost in the photographs were initiating an archegonial branch. The apical cell and the young cells immediately adjacent to it are much larger than the older cells a little farther removed from the apical cell and contain relatively few plastids. In the haematoxylin preparations the cytoplasmic portions of these cells are stained only lightly (text fig. 3).

The more rapid growth of the dorsal portion of the apical region tends to push the apical cell toward the ventral side (fig. 3, Pl. XVI). This tendency is especially noticeable in the greenhouse plants grown from the Florida stock (fig. 10; see also Miss Clapp's fig. 2).


TEXT FIG. 3. Photomicrographs of successive transverse sections of the apical region of the thallus; sections 10 μ in thickness, stained with haematoxylin. × 175.

## DEVELOPMENT OF THE ARCHEGONIUM

The apical cell of an archegonial branch gives rise to relatively few segments, and these branches are consequently so short that it is rarely possible, in examining material *in toto*, to detect any regularity in the arrangement of the archegonia with reference to the axis of the branch. In sections of these branches there appears some evidence to confirm the statements of Miss Clapp for this species, and of Campbell (1905) for the Anacrogynae in general (not including Sphaerocarpales), that each primary segment of the apical cell gives rise to an archegonium. Figures 12 and 13 represent successive vertical longitudinal sections through the short archegonial branch and show the apical cell (*a*), a three-celled archegonium, an archegonium with egg and ventral canal cell formed, and a tangential section of a mature archegonium.

Several thousand archegonia in various stages of development were sectioned and stained in an attempt to obtain the history of the behavior of

the gamete nuclei in fertilization. These preparations afford a favorable opportunity to trace in detail the development of the axial row which seems to be slightly different from that of any of the Hepaticae as yet described in complete detail.

The stages preceding the division of the primary axial cell to form the cover cell and the mother cell of the axial row (terminology of Durand, 1908) have not been examined exhaustively, since these stages are not numerous in my preparations and since the cells in these stages are frequently shrunken somewhat by the action of the fixing reagents. Those cases observed seem to be in harmony with the general account of this development in the Hepaticae and with Miss Clapp's account for this species. I have not attempted to trace the subsequent history of the cap cell, but such observations as I have made seem to indicate that its behavior is so nearly like that of the other wall cells as to make it difficult to distinguish from them.

The mother cell of the axial row grows somewhat before its first division into the central cell and the neck-canal mother cell (figs. 19–22, Pl. XVII). These two cells grow to be relatively large before the next division, which occurs in the neck-canal mother cell (fig. 23) and leads to the formation of an axial row of three penultimate cells (fig. 24). Soon afterward the central cell divides, apparently equally, forming the egg and ventral canal cell (figs. 25–27). This division is followed by a period of growth, especially in the egg (fig. 28). Archegonia at this stage of development are fairly frequent in my preparations. Later the penultimate cell next above the ventral canal cell divides (figs. 29, 30, 38, Pl. XVIII); this division being followed shortly by a division of the uppermost of the penultimate cells of the axial row (figs. 30, 31). The final result is an axial row of six cells—egg, ventral canal cell, and four neck-canal cells. The last two divisions are not frequent in my preparations, and I have not been able to determine whether cell division follows nuclear division in all cases as it seems to in the penultimate cell next above the ventral canal cell shown in figures 29, 30, and 38, followed very soon by a disappearance of the division membrane, or whether the division of the cell may be omitted, as is suggested by such conditions as are represented in figure 31, Plate XVIII, and figures 42 and 43, Plate XIX. Durand (1908) finds that in Marchantia the nuclei of the neck-canal cells sometimes divide just before the disintegration of these cells, these nuclear divisions not being followed by cell divisions. The scarcity of archegonia showing the ultimate cells of the neck-canal row is probably accounted for by the short duration of these cells (Clapp, 1912; Florin, 1918).

I have been unable to detect a cell wall between any two of the cells of the axial row, except usually a thin film between the egg and the ventral canal cell. This film seems to be continuous with the walls of the cells of the venter (fig. 27, Pl. XVII; figs. 32, 34, 35, Pl. XVIII) and stains (with light green) like a cell wall. In disintegrating, the protoplasts of the canal cells probably form a hydrophilous colloidal mass which by its swelling forces open the end of the neck (fig. 33, Pl. XVIII; figs. 39, 40, Pl. XIX).

The cells of the axial row show an alveolar cytoplasmic structure, the alveoli becoming larger as the cells grow older. My preparations in general do not show the plastids clearly, and only rarely can these structures be seen distinctly in any of the cells of the axial row. They are, however, visible in some preparations, both in the cells of the axial row and in fairly young embryos. A few nearly mature eggs show bodies which are probably plastids, and if, as Miss Clapp reports, starch grains are present in the mature egg, there seems little reason to doubt that plastids are present in the egg and zygote throughout their history.

Occasionally, instead of three penultimate cells (central cell and two neck-canal cells) in the axial row at the stage shown in figure 24, Plate XVII, as described above, four are formed. All of these four divide in acropetal succession as do the three of the more frequent type, and this division results in the formation of an egg, a ventral canal cell and six neck-canal cells (figs. 34–37, Pl. XVIII). (See also Clapp, 1912, and Florin, 1918.) In the two archegonia represented in figures 36 and 37, there is no indication of a cell division following the last nuclear division in the neck-canal cells, but the egg and ventral canal cell have evidently begun to disintegrate and these cases cannot be regarded as normal. Axial rows of four penultimate cells are not sufficiently frequent to justify an unqualified statement as to the manner of the formation of the fourth penultimate cell, but the evidence available seems to indicate that the uppermost cell of the three-celled axial row grows and divides before, or at about the same time that, the central cell divides into the egg and ventral canal cell (figs. 34, 35). Another variation of similar type but of inverse order is represented by a single case (fig. 32) in which the usual first division of the neck-canal mother cell was omitted, thus giving rise to only two penultimate cells, one of which has divided to form a typical egg and ventral canal cell and the other is in preparation for division to form a neck-canal row of only two cells.

The exact sequence of divisions in the development of the axial row has not been followed in a large number of the Hepaticae, but certain incomplete accounts seem to indicate that in some of the other Anacrogynae that development is similar to this in Riccardia. Janczewski (1872) described the central cell of *Pellia epiphylla* as remaining undivided while its sister divides to form eight (rarely nine) neck-canal cells. The central cell then divides to form the egg and ventral canal cell, this division being followed by a doubling of the number of cells in the neck and in the neck-canal row, making sixteen (occasionally eighteen) of the latter. He does not say whether the division of the central cell is equal or unequal, nor does he explain the manner of the doubling of the number of cells in the neck-canal row. If, as seems probable, this doubling is brought about by a division of each of the eight (or nine) cells, the egg and ventral canal cell, as in Riccardia, are the oldest of the ultimate cells of the canal row. Hutchinson (1915) reports that the maximum number of neck-canal cells he could find in *Pellia epiphylla*

was nine. It is conceivable that the last division of the neck-canal cells was not shown by his material.

Janczewski reported also that archegonial development in *Fossombronia pusilla* differs from that in Pellia only in the smaller number of neck-canal cells, the central cell dividing when there are four neck-canal cells which later increase to eight (probably by a division of each of the four). This author reported also that "*Jungermannia* [*Lophozia*] *excisa* and *Radula complanata* agree exactly with Fossombronia."

However, he found conditions somewhat different in the Marchantiales. In *Riccia Bischoffii* the axial cell, after the cap cell has been cut off, divides to form the central cell and a neck-canal mother cell. The latter by two successive divisions gives rise to four neck-canal cells, and the former divides, just before fertilization, into a large egg and a small ventral canal cell. In *Preissia commutata*, *Marchantia polymorpha*, *Reboulia hemisphaerica*, *Lunularia vulgaris*, and *Plagiochasma Rousselianum* the process is reported to be similar except that in Marchantia the division of the central cell occurs somewhat earlier, sometimes even before the division of the penultimate cells of the neck-canal row.

Garber (1904) has followed in detail the development of the archegonium of *Riccia natans* and confirms the observations of Janczewski, adding that the central cell grows rapidly during the period before its division.

The detailed account of archegonial development in *Marchantia polymorpha* given by Durand (1908) confirms all points reported by Janczewski and adds that the ultimate neck-canal cells sometimes show a belated nuclear division which is not followed by a division of the cytoplast. Haupt (1921) confirms Janczewski as to the manner of the division of the central cell in Reboulia and as to the number of neck-canal cells present at the time of this division. He finds, however, that these cells (four in number) are later increased to eighteen or twenty.

The more modern studies on members of the Jungermanniales reveal probably a greater variety of conditions than Janczewski suspected, though none of them give the details of the development of the axial row. Humphrey's (1906) work on *Fossombronia longiseta*, although only fragmentary as concerns the archegonium, seems not to accord with the former author's observations on *Fossombronia pusilla*, or with the conditions found in *Riccardia pinguis*. The work of Haupt (1920) on *Fossombronia cristula* does not include the development of the axial row in sufficient detail to admit of a comparison. His earlier work (1918) on *Pallavicinia Lyellii* is more satisfactory in respect to the archegonium. In this species the axial cell is said to divide into a primary neck-canal cell and a primary ventral cell.

The development of the axial row [neck-canal row] usually precedes the division of the primary ventral cell, although frequently mitoses can be seen in the neck cells after the formation of the ventral canal cell and egg. In most cases about ten neck-canal cells were

seen; sometimes, however, as many as eighteen are formed. The primary ventral cell, by a transverse division, produces a ventral canal cell and egg which are almost equal in size.

The ventral canal cell disintegrates very soon after being formed, and its disintegration is followed by that of the neck-canal row.

The most satisfactory account of archegonial development in any of the Jungermanniales (not including Sphaerocarpales) is that of Grün (1914) for *Treubia insignis*. Here the mother cell of the axial row divides, as in probably all the Hepaticae, into a central cell and a neck-canal mother cell.

From the neck-canal mother cell there next arise successively four neck-canal cells. These undergo in the course of development a doubling to eight cells. These latter again divide so that we find in the fully grown archegonium sixteen neck-canal cells. During the formation of the eight neck-canal cells a division is likewise observed in the ventral tier of cells, that is, in the secondary central cell which has previously been changing slowly into a spherical form. This [division of the central cell] results in the formation of a smaller ventral canal cell and a considerably larger spherical egg.

Of the Sphaerocarpales, *Geothallus tuberosus* has been studied by Campbell (1896), *Sphaerocarpos texanus* by Miss Rojas (1918), and *S. Donnellii* by Miss Hartman (1918). All three are of the Riccia type so far as concerns the development of the axial row. The mother cell of the axial row divides into a central cell and a neck-canal mother cell. The latter, by two successive divisions, forms four neck-canal cells, these divisions being followed by an unequal division of the central cell to form the egg and ventral canal cell (the consecutive order of divisions is not stated for Geothallus).

The present state of our knowledge does not justify an extended generalization on the development of the axial row in the liverworts. In all the Hepaticae described (not including the Anthocerotales, which differ only slightly), this row develops from a primary axial cell which divides first into a central cell and a neck-canal mother cell. The latter by successive divisions gives rise to the neck-canal cells whose number is usually a power of two (occasional exceptions as to number in Riccardia, Pellia, Reboulia, and doubtless other forms). In the Jungermanniales studied the division of the central cell precedes the last division of the neck-canal cells (with possible exceptions in Pellia, according to Hutchinson, and in Pallavicinia, according to Haupt). In the Sphaerocarpales there are only two successive divisions of the neck-canal mother cell and its daughter cells, and these are completed before the division of the central cell. In the Marchantiales, the division of the central cell may be followed by one or more divisions in the neck-canal cells, as in Reboulia (Haupt, 1921), or it may occur after the last of these divisions, as in Riccia (Janczewski, Garber), or both conditions may be found in the same species, as in Marchantia (Durand). Which of these conditions is the more primitive, one can only conjecture. If we assume that the condition characteristic of the Jungermanniales is the more primitive, we might conclude that the last division of the neck-canal cells has been suppressed in the Sphaerocarpales and in

the simpler Marchantiales. A possible basis for such an assumption is afforded by Marchantia (Durand), in which the four neck-canal cells sometimes become binucleate as though preparing for this suppressed division; but this fact might also be interpreted in a converse manner to support the assumption that the condition in the Sphaerocarpales is the more primitive one, and that the Jungermanniales and the higher Marchantiales have acquired the habit of additional divisions in the neck-canal cells. It has not been shown whether or not environmental conditions may affect the number of divisions in the neck-canal cells. Hutchinson found only half as many neck-canal cells in *Pellia epiphylla* as did Janczewski. This reduction may conceivably have resulted from a suppression of the last division due to the influence of the environment in which his plants had grown.

## Non-functional Archegonia

Abnormal sex organs are generally recognized as occurring frequently in the Bryophyta, and Florin (1918) has described several abnormal archegonia in *Riccardia pinguis*. One of these is an archegonium with a single egg and a double canal row. In my material I have found one archegonium which may be considered a complementary case. In this archegonium there are two eggs and two ventral canal cells, but only one row of neck-canal cells (fig. 38, Pl. XVIII). One egg and one ventral canal cell form the usual part of an apparently normal axial row. The additional egg and ventral canal cell appear slightly lower down in the archegonium and are separated from those of the complete axial row by a film which seems to be a cell wall. This archegonium is considerably more massive than those of the usual type, and a rift appears between the wall proper and the cells immediately above the supernumerary ventral canal cell. I have not been able to determine how far this rift extends around the column of cells above the ventral canal cell. The cells of this column have angular walls and numerous plastids and present the appearance characteristic of cells of the archegonial wall, with which the column is continuous above.

In the locations from which my material was collected Riccardia produces large numbers of archegonia, of which only a small percentage function in reproduction. It is apparent from the sections that a large percentage of the mature archegonia in the material collected were not capable of so functioning. In many cases it appears that disintegration has begun in the egg and the ventral canal cell before the maturity and normal disintegration of the neck-canal cells (figs. 36, 37, Pl. XVIII; figs. 42–46, Pl. XIX). Those in which this disintegration appears to be well advanced stain deeply with safranin although the cytoplasm of the neck-canal cells does not take this stain (figs. 36, 37, Pl. XVIII; and figs. 42, 45, 46, Pl. XIX). This premature disintegration of the egg and ventral canal cell is generally accompanied by a marked shrinkage of these cells, and the later swelling of the neck-canal cells sometimes causes the latter to push down into the venter (fig. 44).

Another type of disintegration frequently found is apparently due to the failure of the egg to be fertilized. Although the time during which the egg is capable of functioning as a gamete is probably short, the process of disintegration seems to be relatively slow. One sometimes finds the egg nucleus easily recognizable after the protoplasts have disappeared from the other cells of the archegonium. In the case shown in figure 40, the cytoplasm of the egg is represented only by a droplet of non-staining liquid in which are suspended globules of deeply staining material, while the nuclear membrane and nucleolus seem to be intact. Figure 39 represents an early stage of disintegration in which the cytoplasm still shows some of its alveolar structure but takes the basic stain.

Disintegration is also found to occur even after the first segmentation of the fertilized egg. Figure 41 represents a disintegrating embryo of two cells in an archegonium from the cells of whose wall all protoplasmic contents have disappeared. In this case the conditions inhibiting normal development probably obtained before the segmentation of the zygote, which has not elongated into the typical haustorial cell and epibasal cell (fig. 14, Pl. XVI; see also Miss Clapp's fig. 34).

The striking resemblance of many of these disintegrating cells to the figures shown by Florin and Miss Clapp arouses some doubt as to whether the cells figured by these workers were in all cases functional eggs. I have not found a case exactly similar to the one shown by Florin in his figure 1, but such a case would be comparable to some of those figured in the present paper. He says:

> In one archegonium [his fig. 1] I found four cells in a row, all morphologically and probably physiologically equivalent and supplied with large nuclei and deeply staining cytoplasm. They had also the appearance normally possessed only by the egg.

In view of the conditions found in my material, I should rather suspect that the archegonium represented in his figure is one in which disintegration had begun simultaneously in all four of the axial cells of about the stage shown in my figures 27 and 28, Plate XVII. Certainly the cells figured show little resemblance to the functional egg (fig. 33, Pl. XVIII). Miss Clapp's figure 33 is much more nearly typical, but probably represents an early stage of disintegration due to the lack of fertilization such as is represented in the present paper by figure 39, Plate XIX.

No attempt has been made to follow the sequence of cell divisions in the growth of the wall of the archegonium. These divisions appear to be quite irregular and frequent until the time of fertilization and afterward.

The mature archegonium is a massive structure, and, as described by Miss Clapp, its wall, except for a very small terminal portion, has two layers of cells (figs. 30–33, 35, 36, Pl. XVIII; figs. 42–50, Pl. XIX; Miss Clapp's figs. 32, 33). The neck of the archegonium is not made up of five longitudinal rows of cells as described by Janczewski for the Jungermanniales

studied by him, but shows in transverse section many more than that number. Figure 47 shows a cross section of the neck of a nearly mature archegonium at a distance of about 20 $\mu$ from its tip. Figure 48 shows a similar section of the same archegonium at a distance of about 50 $\mu$ from the tip. Figure 49 shows the cross section of the same archegonium just above the venter and about 80 $\mu$ from the tip. Figure 50 shows the section through the egg and venter at a distance of about 110 $\mu$ from the terminal end.

The development of the embryo seems to furnish a stimulus to very rapid growth and division in the cells of the venter as well as in those of the thallus immediately below (figs. 14–17, Pl. XVI). This results in the early formation of a massive "calyptra," of which only the upper portion has been derived from the archegonium (figs. 17, 18; see also MacVicar, 1912, p. 50, fig. 1). The cells of the thallus in close proximity to the young embryo contain a large number of chloroplasts and are doubtless active in metabolism. As observed by Miss Clapp, rhizoids arise from the basal portion of the "calyptra" and probably help to supply water and mineral nutrients for the increased metabolic activity.

According to Miss Clapp, the first segmentation of the fertilized egg is tranverse and divides the zygote into a hypobasal cell, which elongates into a haustorium, and an epibasal cell which gives rise to the embryo proper. Only three two-celled embryos have been found in my preparations, and none of these appears to have been alive or growing when fixed. The one shown in figure 41, Plate XIX, was sectioned obliquely, but it is obvious that it was in a degenerating condition; it had not elongated, the cytoplasm of the cells shows no definite organization, and all the protoplasmic content has disappeared from the cells of the wall of the archegonium. Figure 14, Plate XVI, shows another two-celled embryo whose haustorium is fairly well developed, but whose epibasal cell had apparently begun to disintegrate; the nucleus is scarcely recognizable, and the cytoplasm is disorganized. The third two-celled embryo, not figured, is intermediate between these two, both as to the amount of elongation and as to the degree of preservation of its protoplasmic content.

The youngest apparently normal embryo found is shown in figure 15, Plate XVI; it consists of three cells, a haustorium of one cell and the embryo proper of two cells. In this case the division of the epibasal cell was in a plane at right angles to the first division of the zygote. Figure 16 represents a longitudinal section of a nine-celled embyro; the embryo proper consists of a regular octant, of which two cells adjacent to the haustorium are in telophase. Figure 17 shows a later stage, in which the haustorium has reached its full size and in which the capsule can be distinguished from the seta. Figure 18 shows in outline the mature sporophyte within the massive "calyptra."

Miss Clapp describes the first three divisions in the zygote as horizontal, resulting in the formation of a filament of four cells, including the haus-

torium. My observations on these early stages do not accord with hers, but are too fragmentary to justify a questioning of her results.

## Summary


1. *Riccardia pinguis* is a cosmopolitan species whose morphological features very somewhat under different environmental conditions.

2. The species is probable dioecious, but the male and female thalli are distinguishable only by their reproductive branches or by the presence or absence of sporophytes.

3. The spores when discharged from the capsule do not ordinarily adhere in tetrads, but the tetrads may be preserved by proper manipulation.

4. The growth of the thallus is apical, by means of a "two-faced" apical cell which gives rise to segments alternately from its right and left cutting faces.

5. The early divisions of the primary segment of the apical cell follow the scheme of Leitgeb (1877).

6. The axial row of the archegonium develops from a mother cell which divides to form a central cell and a neck-canal mother cell; the latter divides once, increasing the axial row to three penultimate cells which divide in acropetal succession forming ultimately an egg, a ventral canal cell, and four neck-canal cells. Occasionally, the number of penultimate cells is four instead of three and the ultimate number of neck-canal cells six instead of four.

7. Disintegrating archegonia are numerous in the material used for this study; the egg and ventral canal cell frequently break down before the maturity and disintegration of the neck-canal cells.

8. The young embryos found in this material show an order of division slightly different from that reported by Miss Clapp (1912).

9. A massive "calyptra" is formed around the sporophyte by the rapid growth and division of the cells of the venter and of the cells of the thallus immediately below the archegonium.


I wish to acknowledge my gratitude and indebtedness to Dr. W. N. Steil, who gave valuable assistance in procuring material; to Mr. Severin Rapp of Sanford, Florida, who supplied living plants from his locality; to Professor A. W. Evans of Yale University, who confirmed the identification of all material used and gave valuable suggestions; and especially to Professor C. E. Allen, under whose guidance these studies were made.

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## EXPLANATION OF PLATES

All figures were drawn from stained sections with the aid of an Abbé camera lucida, using Leitz objectives and oculars, and were reduced in reproduction to the magnifications indicated; Plate XVI being reduced three fifths, Plates XVII–XIX one half.

### Plate XVI

Fig. 1. Horizontal section of a part of the apical region of the thallus showing the apical cell (*a*) in process of division. × 160.

Fig. 2. Vertical longitudinal section of secondary segments (*b′* and *c*) formed by the division of a primary segment; serial section adjacent to and following the one shown in figure 3. × 160.

Fig. 3. Median vertical longitudinal section of the apical region of a vegetative branch of the thallus showing apical cell (*a*). × 160.

Figs. 4–8. Successive serial transverse sections of the apical cell (*a*), and of the adjacent cells in a vegetative branch; *b′* and *c* at the right in figure 4 probably represent the anterior ends of cells formed from the youngest segment of the apical cell; *c*, *d*, *e*, and *e′* at the left in the same figure probably represent the anterior ends of the forward tier of cells formed from the second youngest segment. × 160.

FIG. 9. Vertical longitudinal section through the primary segment (*b*) in process of its first division; serial section adjacent to and preceding the one shown in figure 10. Greenhouse plant of Florida stock. × 160.

FIG. 10. Median vertical longitudinal section of apical end of vegetative branch showing apical cell (*a*). Greenhouse plant of Florida stock. × 160.

FIG. 11. Vertical transverse section of apical cell (*a*, *b*) in process of division; *b* represents the portion which would have become the segment. Greenhouse plant of Florida stock. × 160.

FIGS. 12, 13. Successive vertical longitudinal sections of a young archegonial branch; figure 12 shows the apical cell *a*, figure 13 the secondary segments *b′* and *c* and a three-celled archegonium (♀). × 230.

FIG. 14. Longitudinal section of archegonium and two-celled embryo (probably not functional); reconstructed from two adjacent sections. × 230.

FIG. 15. Longitudinal section of archegonium and three-celled embryo; reconstructed from four serial sections. × 230.

FIG. 16. Longitudinal section of nine-celled embryo and gametophyte tissue enlarging to form the massive calyptra; *V*, ventral surface of thallus; *D*, dorsal surface; reconstructed from four serial sections. × 116.

FIG. 17. Longitudinal section of young calyptra and young sporophyte; *V*, ventral surface of thallus; *D*, dorsal surface; reconstructed from three serial sections. × 116.

FIG. 18. Outline of longitudinal section of mature calyptra and nearly mature sporophyte; reconstructed from two sections not adjacent. Greenhouse plant of Wisconsin stock grown in 1920. × 20.

PLATE XVII

Longitudinal sections of archegonia.

FIG. 19. Very young archegonium showing the mother cell of the axial row. × 950.

FIG. 20. Mother cell of axial row in early prophase of division. × 950.

FIG. 21. Mother cell of axial row with division almost completed. × 950.

FIG. 22. Central cell and neck-canal mother cell formed by first division. × 950.

FIG. 23. First division of neck-canal mother cell in progress. × 950.

FIG. 24. Axial row of three penultimate cells, the central cell and two neck-canal cells. × 618.

FIG. 25. Early prophase of division of the central cell. × 618.

FIG. 26. Late telophase of division of the central cell. × 375.

FIGS. 27, 28. Axial row of four cells—egg, ventral canal cell, and two penultimate neck-canal cells. × 375.

PLATE XVIII

FIG. 29. Lower penultimate neck-canal cell in process of division. × 375.

FIG. 30. Axial row consisting of five cells—egg, ventral canal cell, two ultimate neck-canal cells, and the upper penultimate neck-canal cell, the latter in prophase of division. × 375.

FIG. 31. Mature axial row consisting of egg, ventral canal cell, and two binucleate neck-canal cells; reconstructed from three serial sections. × 375.

FIG. 32. Axial row consisting of egg, ventral canal cell, and one penultimate neck-canal cell; reconstructed from two sections. × 375.

FIG. 33. Mature archegonium with egg ready for fertilization, antherozoid present in venter; reconstructed from two sections. × 375.

FIG. 34. Axial row consisting of egg, ventral canal cell, and three penultimate neck-canal cells; reconstructed from four serial sections. × 375.

FIG. 35. Similar to figure 34, except probably a little older; reconstructed from two sections. × 375.

FIG. 36. Axial row consisting of egg, ventral canal cell, one binucleate neck-canal cell, and two penultimate neck-canal cells in process of division; egg and ventral canal cell disintegrating; reconstructed from three serial sections. × 375.

FIG. 37. Mature axial row with three binucleate neck-canal cells, all markedly shrunken; egg and ventral canal cell badly disorganized. × 375.

FIG. 38. Archegonium with two eggs, two ventral canal cells, and one neck-canal row; reconstructed from three serial sections. × 375.

## PLATE XIX

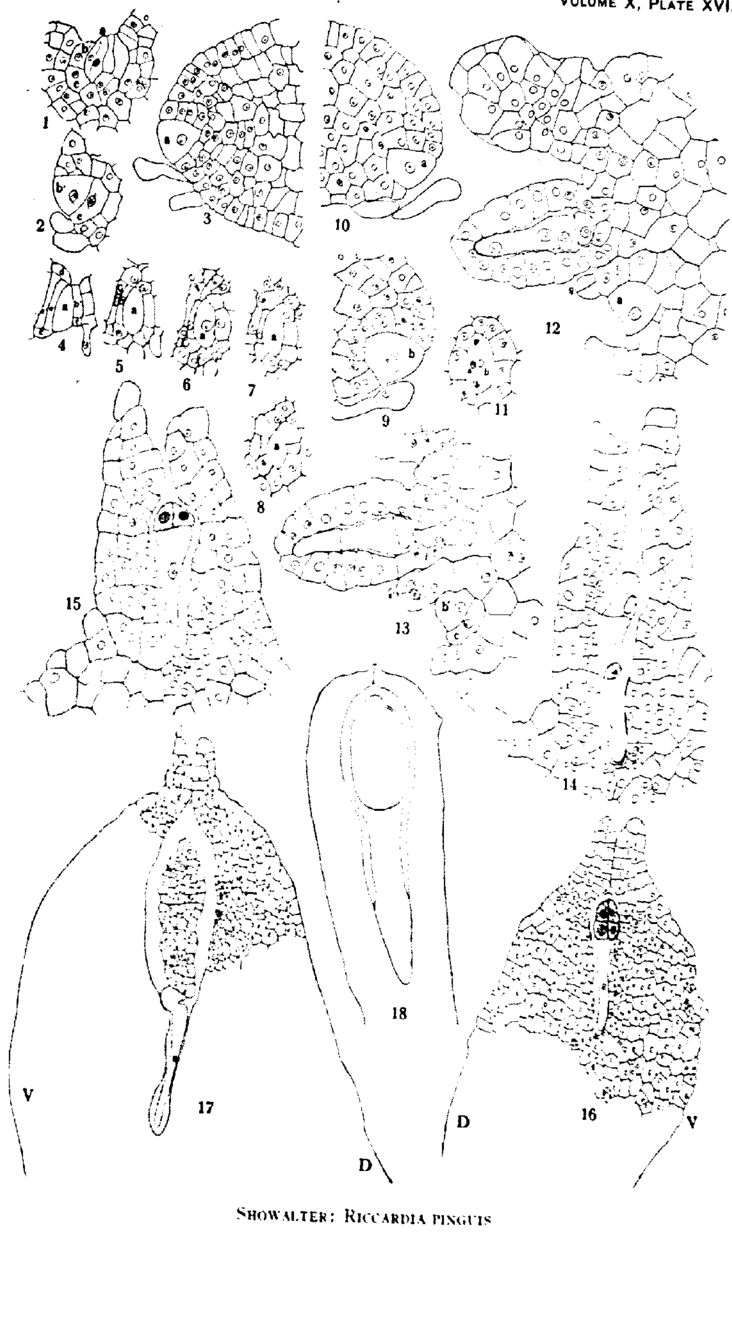
FIG. 39. Longitudinal section of archegonium showing egg in early stage of disintegration due to failure to be fertilized. × 375.

FIG. 40. Archegonium showing late stage of disintegration; no protoplasm visible in cells of wall but nucleus of egg still recognizable. × 375.

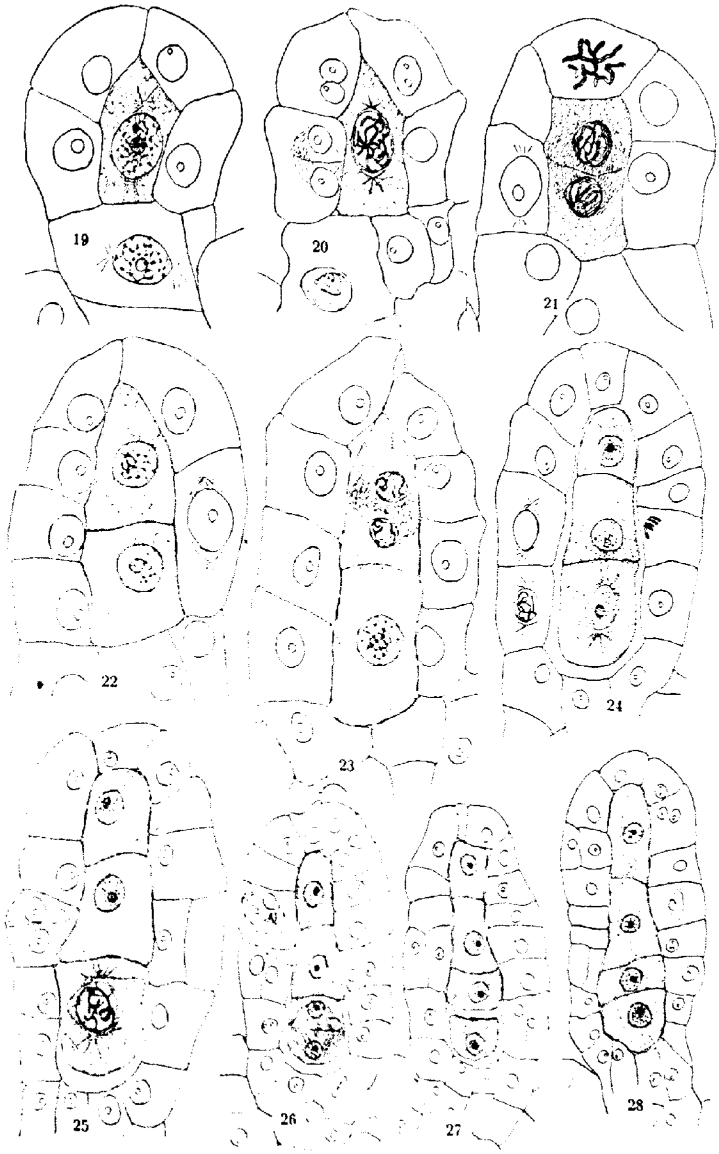
FIG. 41. Oblique section of archegonium and two-celled degenerating embryo; no protoplasm in cells of archegonial wall. × 375.

FIGS. 42–46. Premature disintegration of egg and ventral canal cell; figure 45 reconstructed from two sections. × 375.

FIGS. 47–50. Transverse sections of a mature archegonium; figure 47, third serial section from terminal end; figure 48, sixth serial section; figure 49, ninth serial section, just above the venter; figure 50, twelfth serial section, through venter and egg. × 200.




SHOWALTER: RICCARDIA PINGUIS




SHOWALTER: RICCARDIA PINGUIS

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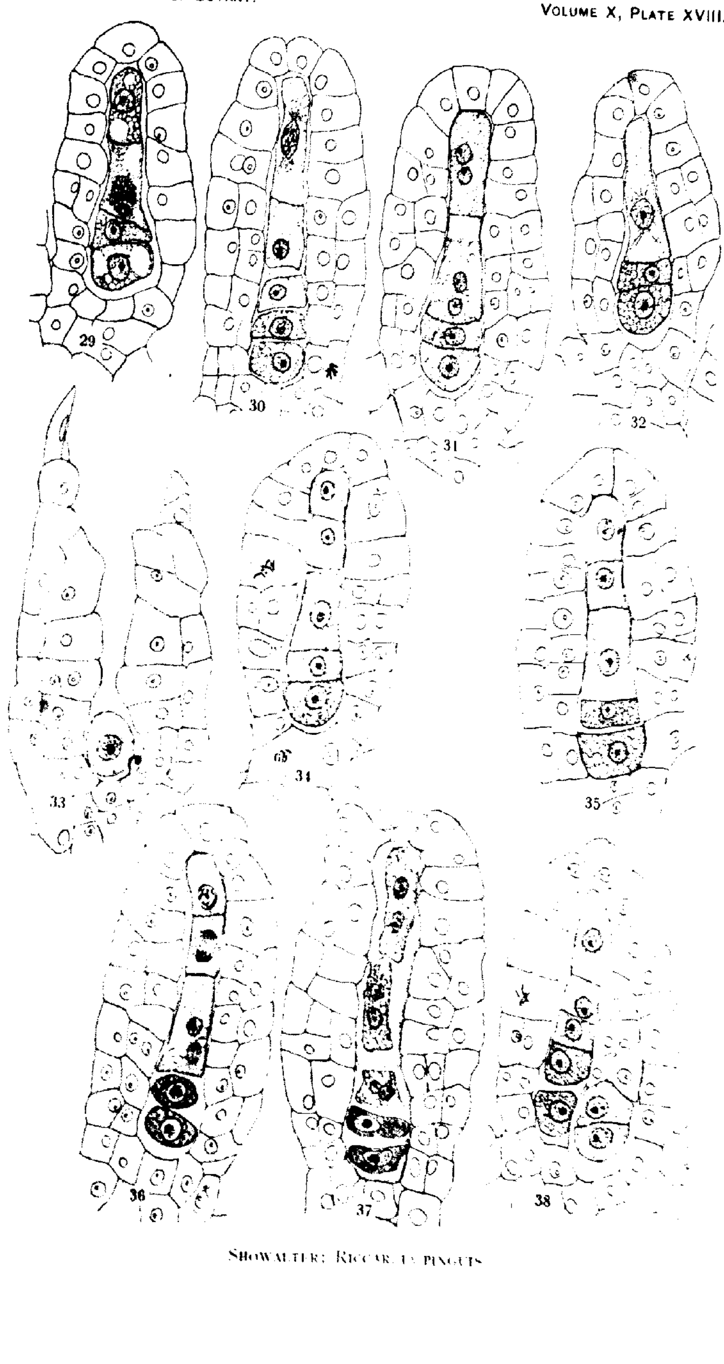
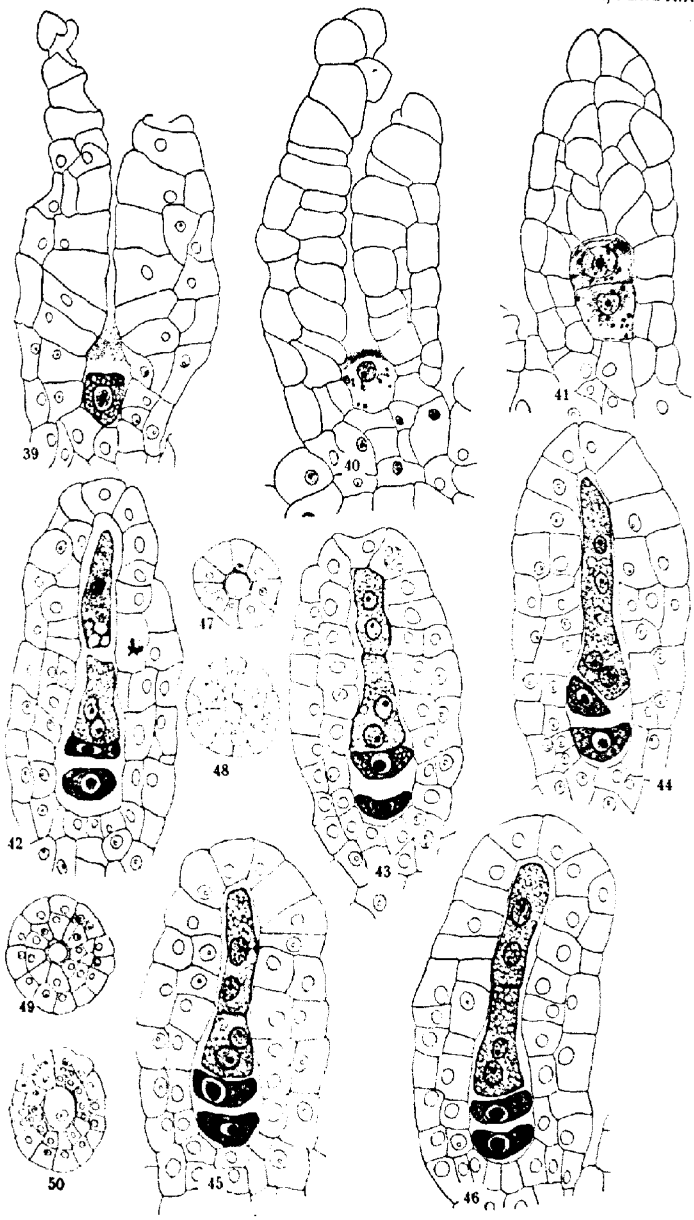



SHOWALTER: RICCARDIA PINGUIS




SHOWALTER: RICCARDIA PINGUIS

## Methods of Experimentation

Two species of Rhizopus, *R. tritici* and *R. nigricans*, were employed. These two species were selected because among the parasitic species they represent the two extremes in the amount of pectinase produced. *Rhizopus tritici*, under the conditions of the writers' previous experiments, secreted a very active macerating enzym while that secreted by *R. nigricans* was much less active.

The spores were obtained in the following manner: Several 2-liter flasks containing about 500 cc. of sweet-potato decoction were inoculated with the organisms under investigation. At the end of ten days or two weeks spores were abundantly produced. The fungous felt was then carefully lifted from the flasks, the lower side of the felt was held under the tap for a few moments to wash away the decoction, and then the felt was floated, the upper side down, in a vessel of distilled water. By gently agitating the fungous mat a considerable quantity though not all of the spores were removed. The spore suspension was then filtered through a good grade of muslin, about 27 threads to the centimeter, to remove any fungous threads that might have broken off during the process of removing the spores. At this stage a microscopic examination of the suspension was made, and if any bits of mycelium were found in the solution it was either filtered again or discarded. When the suspension was free of fungous *débris* the spores were caught on a number 2 chemically prepared Whatman filter paper. The spores were then washed from the filter paper with acetone. They were exposed to the acetone for 10 minutes, then caught on a tarred filter paper, and finally treated with ether by pouring the latter on to them on the filter paper. The filter paper was then removed from the funnel, straightened out, and stored away in any suitable container until required for use. Tests have shown that spores so treated will not germinate.

After the dry weight of the spores was obtained, their macerating action on raw sweet-potato discs was determined by immersing the latter in a water suspension of the spores. At this point no attempt was made to separate the spores from the paper, the latter being included in the system. The amount of water used was determined by the weight of the spores. Ten cubic centimeters of water were used for every 0.10 gram of spores. Since it was impossible by the methods employed to obtain a definite weight of spores, the raw discs were exposed to a spore suspension of fairly uniform density by this adjustment of the relationship of the water and spores.

## Experimental Data

Several experiments were conducted with the spores of both *Rhizopus tritici* and *R. nigricans*. In all cases except a few of the preliminary ones, the actual weight of the spores was determined. In every case one control

was held in which the spore suspension was heated in order to inactivate the enzym. Maceration was carried out at a temperature of 35° C. A little toluol was added to each flask to prevent the growth of contaminating bacteria.

The enzym was relatively dilute, so that maceration progressed rather slowly. No maceration occurred in the control flasks. In the other flasks the discs became somewhat crisp at first but later became flaccid. This stage was then followed by a dissolution of the middle lamellae, which was typical in every respect of that produced by a water extract of the mycelium or by the solution on which the fungus grew.

The rate of the maceration by the enzym produced by the spores of these two species differed considerably. The action by *R. tritici* was relatively rapid, the discs being completely macerated in 24 to 48 hours. *Rhizopus nigricans*, on the other hand, acted very slowly, and in some cases no maceration took place in 72 hours. The writers have found also that the enzym per unit volume of solution or per unit weight of mycelium is much weaker in *R. nigricans* than in *R. tritici*. Without going further into the details of these experiments or their results, the evidence conclusively shows that the spores of both species contain an enzym which is able to cause disintegration of the middle lamellae of raw sweet potatoes.

It is very likely that this enzym plays an important rôle in the early nutrition of the fungus and that it may be a factor in the initial infection of some of its hosts.

## Summary

The spores of *Rhizopus nigricans* and *R. tritici* both contain an enzym, pectinase, which is capable of dissolving the middle lamellae of raw sweet potatoes. The rate of maceration by the spores of *R. nigricans* is relatively much slower than that produced by the spores of *R. tritici* when the concentration of the spores by weight is the same.

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# THE CHROMOSOMES OF *RICCARDIA PINGUIS*

Amos M. Showalter

(Received for publication June 20, 1922)

This study of the chromosomes of *Riccardia pinguis* (L.) S. F. Gray was made with reference primarily to possible sex differences. The material used was collected August 11 and 12, 1921, in the swamp prairie bordering Lake Waubesa, near Madison, Wisconsin. The methods of fixation, staining, etc., have been reported in a previous paper (Showalter, 1923) and need not be repeated here. This material was supplemented with greenhouse plants grown from Florida and Wisconsin stock.

My choice of this plant for such a study was due in part to a suggestion of Dr. W. N. Steil, who had observed that the male plants are sometimes noticeably smaller than the female, but my observations in field and cultures have convinced me that there is no marked sexual dimorphism in this species.

Division figures are fairly frequent in the massive embryonic tissue at the growing ends of the thallus and in the young sex organs. The chromosomes are relatively large and are easily stained so that they stand out in brilliant color contrast to the rest of the cell contents. The spermatogenous cells of the antheridium, however, are less favorable for counts of the chromosomes because the cells are small (except in very young antheridia) and division figures are consequently crowded, so that the chromosomes of one cell are not easily distinguished with certainty from those of the adjacent cells.

In the cells of the embryonic tissue of both male and female plants and in those of the archegonium, the chromosomes are frequently well spread out on the spindle at the equatorial plate stage and in polar view are especially favorable for study. I have studied a large number of division figures in the plants from the region of Madison, and the evidence seems conclusive that the haploid number of chromosomes is ten (figs. 1–18, Pl. XX). Farmer (1905), in studying the reduction divisions, reported casually that the number "seems to be eleven for the species in question." Occasionally a small spherical body which stains like a chromosome is found near or among the chomosomes (figs. 2, 11, 12). I have not traced the behavior of this body, but there is little reason to suspect that it may be chromosomal in nature or origin. It may possibly be a fragment or vestige of the nucleolus.

The chromosomes are relatively smooth, rod-shaped structures, fairly uniform in thickness and variously bent (figs. 1–19). They differ somewhat in size, but only one, the smallest, is distinguishable with certainty in any large number of cases. The chromosomes which appear in sporo-

phytic divisions have the same general appearance as have those seen in the thallus, though, of course, in the double number (fig. 19).

I have not studied exhaustively the chromosomes of the plants received from Florida, but the few dozen division figures examined have convinced me that the number and relative sizes of the chromosomes are the same as those of plants from the vicinity of Madison (figs. 20–24). In two or three division figures from the Florida plants there appear to be eleven chromosomes (fig. 21), but this appearance may be due to a part of one chromosome having been displaced by the microtome knife.

Previous investigators of the morphology of this species agree that it is strictly dioecious, but this conclusion seems to be based upon field observations and not, so far as I have been able to find, upon conclusive cultural experiments. My own attempts to grow the plant in culture have not been highly successful and have yielded no conclusive evidence, but in all cases observed, both in cultures and in the field, the male and female sex organs were produced on separate plants.

The comparative study of the chromosomes of the two sexes, as in the case of my earlier study of the chromosomes of Conocephalum (Showalter, 1921), has revealed no perceptible difference between the sexes. Exact micrometric measurements of the chromosomes have not been made, but the camera lucida drawings show no perceptible difference between the chromosomes of the male and those of the female plants (figs. 1–18). The evidence seems conclusive that there is in this species no such chromosome difference between the sexes as is found in Sphaerocarpos (Allen, 1917, 1919; Schacke, 1919).

I wish to express my gratitude to Professor C. E. Allen, under whose direction this study was made.

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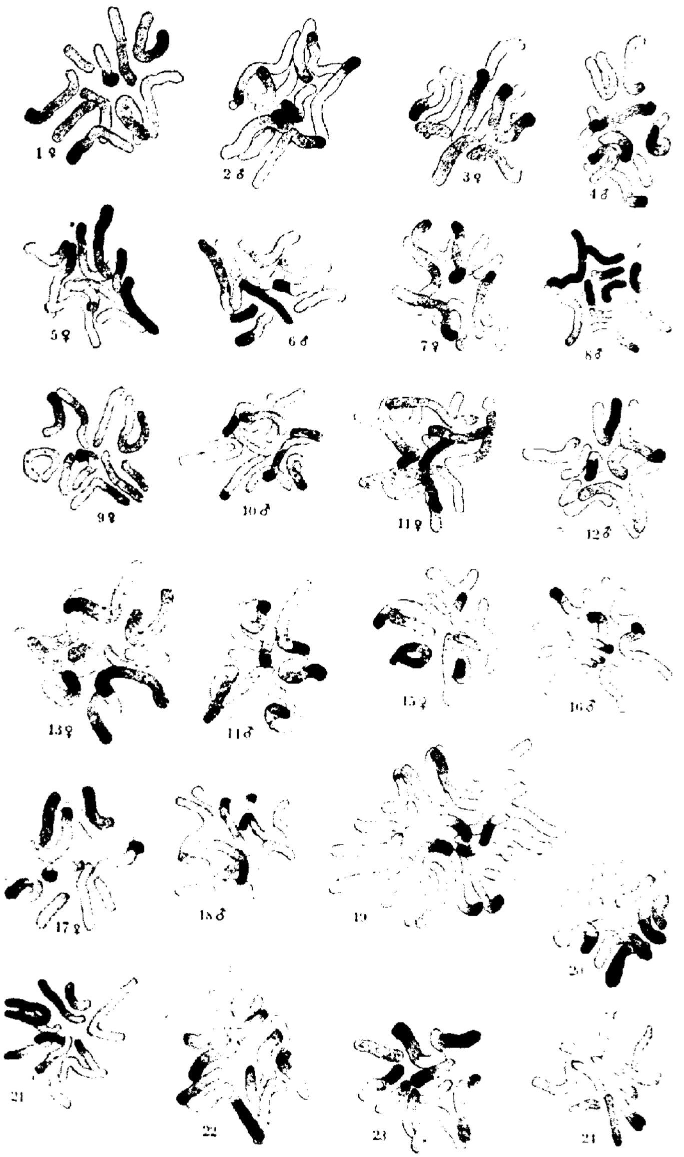
## EXPLANATION OF PLATE XX

All figures were drawn with the aid of an Abbé camera lucida, using a Zeiss 2-mm. apochromatic objective N. A. 1.40, and compensating ocular 18, at a magnification of about 4,000 diameters; reduced in reproduction to about 2,650.

FIGS. 1–18. Chromosome groups, alternately from female and male plants (Wisconsin stock), as indicated by symbols: 1, from cell of dorsal surface layer of thallus; 2–8, from cells of interior of thallus; 9, from cell of wall of archegonium; 10, from cell of dorsal surface layer; 11, from cell of wall of archegonium; 12, from cell of interior of thallus; 13, from cell of papillate scale; 14, from cell of dorsal surface layer; 15, from cell of ventral surface layer; 16, from cell of interior of thallus; 17, from cell of dorsal surface layer; 18, from cell of ventral surface layer.

FIG. 19. Chromosomes in cell of capsule wall, from greenhouse culture of Wisconsin stock.

FIGS. 20–24. Chromosomes of greenhouse plants grown from Florida stock: 21, 22, from cells of interior of thallus; 23, from cell of ventral surface layer; 24, from cell of dorsal surface layer.




SHOWALTER: CHROMOSOMES OF RICCARDIA

# AUSTRALASIAN BOTANICAL NOTES

## II. VICTORIA, SOUTH AUSTRALIA, AND WEST AUSTRALIA

Douglas H. Campbell

(Received for publication July 11, 1922)

### Victoria

Victoria, the smallest of the Australian States except Tasmania, has a rather more uniform climate, both as to temperature and rainfall, than most of Australia, and in this respect more nearly resembles certain regions of the north-temperate zone. Victoria occupies nine degrees of longitude and five of latitude, its southernmost point, Wilson's Promontory, extending just beyond the thirty-ninth parallel. In area Victoria is 87,884 square miles, a little more than the area of Kansas. The eastern portion, Gippsland, is a continuation of the coastal belt of New South Wales, and to the north of this is an elevated region, which is a continuation of the main mountain mass of New South Wales. Within Victoria this is known as the Victorian Alps. In the southwest there is an extensive basaltic plain of great fertility, one of the most productive regions in the commonwealth.

The northwest corner of the state is a continuation of the interior plains of New South Wales and South Australia, and like them is a region of scanty rainfall.

The heaviest rainfall is in parts of Gippsland, where some stations have over sixty inches, and nearly a third of the state has a rainfall exceeding thirty inches.

In these well-watered regions, especially in the mountainous parts of Gippsland, there is a heavy forest, mainly Eucalyptus. It is in the Gippsland forest that the giant among Australian trees is found. This is *Eucalyptus regnans*, which is a close rival in height of the Californian Sequoias.[1]

Victoria has proportionally a larger amount of land available for ordinary agriculture than any of the other states, and in consequence is more uniformly populated and looks more like the agricultural regions of Europe and America.

Melbourne is in many ways the finest of the Australian cities. Its broad, well-kept streets and handsome and substantial public buildings and business structures give the impression of a remarkably prosperous community.

[1] Maiden, in his sketch of the Australian flora (Federal Handbook, p. 204), states that the "official size" of the tallest Gippsland tree was 326 feet 1 inch height, and girth 25 feet 8 inches, six feet from the ground.

As in the other large cities, ample provision has been made for parks, including an uncommonly attractive and interesting botanical garden. Close to the gardens and the adjacent park lands is the river Yarra, which flows through the city. From the river an ample supply of water for the gardens is available, and this, together with good soil, gives the gardens a great advantage over those of Sydney, and this shows especially in the fine stretches of lawn and the luxuriant growth of many deciduous trees and shrubs which do not thrive in Sydney.

It is true that the colder winters of Melbourne are unfavorable to the growth of strictly tropical and many subtropical species; but this is counterbalanced by the much better growth of the plants of more temperate regions. Trees and shrubs from temperate America, Asia, and Europe do much better in Melbourne than in Sydney or Brisbane, and the same is true of the hardy bulbs and other spring flowers.

In September and October the gardens were looking very beautiful. The early flowering trees and shrubs, especially the Japanese cherries, double-flowering peaches, Magnolias, and Judas tree, were unusually good and made a brilliant display. Camellias and Indian Azaleas were also very fine, although the latter were perhaps not quite so luxuriant as in Sydney. There were also some good Rhododendrons. Where they were sheltered, several species of tree ferns and palms grew very well. A particularly satisfactory effect was attained in one place, where, under the shelter of large trees, there was a fine plantation of tree ferns and palms which looked very tropical. The tree ferns comprised several species of Cyathea, Dicksonia, and Alsophila, the finest being *A. excelsa* from Norfolk Island. The palms included the two native species, *Archontophoenix Cunninghamii* and *Livistona australis*, and the New Zealand *Rhopalostylis sapida*.

I was especially interested in an excellent collection of American trees, which seemed very much at home. These included very fine specimens of several oaks—*Quercus alba*, *Q. rubra*, *Q. coccinea*, and one or two others—Liquidambar, Taxodium, Robinia, Gleditschia, *Acer rubrum*, and *Cornus florida*. The latter was in flower, but the flowers were not abundant.

A large and well-labeled collection of Australian plants, including many of the very showy West Australian flowers, is an important feature of the garden. Among the more striking of these were flowering specimens of the great torch lilies (*Doryanthes excelsa*, *D. Palmerstoni*) and the beautiful scarlet gum, *Eucalyptus ficifolia*, from western Australia.

Because of the limited time at my disposal, I was able to make only two botanical excursions while in Victoria. Owing to its smaller size and more uniform climate, Victoria has a much smaller proportion of peculiar species than the larger states, comparatively few species being confined to it.

In the well-watered eastern sections there is a heavy forest, but only a small number of the Malayan types characteristic of the rain forests of Queensland and New South Wales extend into Victoria. In the extreme

north the palm *Livistona australis* is said to grow, and a few other subtropical genera occur; but for the most part the forest is composed of various species of Eucalyptus, of which the giants are *E. regnans* and *E. globulus*, the Tasmanian blue gum, so extensively planted in California and elsewhere.

On a former visit to Australia, in 1903, I made a brief trip to the Black Spur, in the Gippsland mountains, and saw some very fine specimens of the giant gums. This forest is a very beautiful one, as among the great trees in many places are groves of tall tree ferns. As much of the forest has been destroyed since my visit, one wonders whether any of the biggest of these trees are still standing.

The tree ferns are largely *Dicksonia antarctica*, but *Alsophila australis* also occurs. There are some interesting bryophytes also common in this region, notably the giant moss, *Dawsonia superba*, and the liverwort *Umbraculum flabellatum*.

A visit, in company with Professor Ewart of Melbourne, was made to this region, but at a considerably lower elevation; and as most of the forest had suffered greatly from fire, and the region as a whole seemed to be much dryer, the vegetation was decidedly less interesting. The forest trees were for the most part much smaller than on the Black Spur, and the ferns and liverworts were less abundant. Several pretty orchids were seen, and some interesting heaths and sundews. The latter, together with some of the orchids, were especially abundant where the ground had been recently burned over.

The most extensive excursion made in Victoria was to the National Park, in the extreme southern part. Wilson's Promontory, the southernmost point of Australia, has been reserved as a sanctuary for the native plants and animals, and is admirably placed for this purpose. One of the trustees of the park, Mr. Kershaw, accompanied me, and proved a most efficient and entertaining guide. The days spent in his company in the park are among the pleasantest recollections of my stay in Australia.

To reach the park one has to go about one hundred miles by rail, and then one may go part way by motor; but the rest of the way along the sea beach, for two or three hours, was done in a light buggy. On our return we crossed the inlet between the promontory and the mainland in a motor boat, but this is feasible only at high tide.

Going by land, one drives over roads that are by no means perfect—indeed, we were held for an hour or more by our motor being bogged—through low-lying land, much of it a bog. A good many flowers, expecially some pretty heaths, were noted, but no very careful observations of the flora were made. The shore drive was along a barren coast, with sand dunes as we approached the park.

The park comprises about 100,000 acres of mountain and forest, and occupies the greater portion of the peninsula. The isthmus connecting it with the mainland is only about seven miles wide, and across this neck of land there is a strong rabbit- and vermin-proof fence, which completely

shuts off the park and prevents either entrance to or egress from it. Here one may see at large, as nowhere else in Australia, many of the most remarkable members of Australia's strange and interesting fauna.

As we drove along the beach, great black swans rose from the lagoons and flew away, and in other places shore birds of various sorts ran across the beach at our approach. Within the park we found the swans and other water fowl very common. Of the birds, however, none were quite as strange as the emus, a flock of which frequented an open meadow not very far from the rest house where we stayed. Blue and scarlet parrots, and white cockatoos, as well as many other less striking birds, were not uncommon. Kangaroos, which were not at all rare, were seen, and the so-called native bear, a most amusing little animal, resembling exactly the "Teddy bears" of the toy shops.

The flora of the park, owing to the diversity of soil, moisture, and elevation, is a rich one. Along the coast are rocky promontories, extensive beaches, and sand dunes, on which were growing Mesembryanthemums and other dune plants, which were not collected.

About the rest house was open grassland, which was bordered by extensive, dense thickets of *Melaleuca* sp. covering large tracts of swamp land. The lower hill slopes were covered with a thicket of mixed shrubs, much like the chaparral of our Californian hillsides. The most conspicuous member of this scrub was *Leptospermum laevigatum*, then in full bloom and very pretty. The largest members of this association were small trees of *Casuarina* sp., and other shrubs were *Melaleuca* sp., *Acacia dealbata*, *Exocarpus* sp., and others.

The dryer ground was occupied by an open forest of gums, but with these were many Banksias, comprising three species, the commonest being *B. serrata* and *B. integrifolia*. Both of these were trees of fair size, and with them were associated Acacias, Casuarinas, and Hakeas.

The open spaces supported groves of grass trees (*Xanthorrhoea* sp.), their stout flower spikes rising eight or ten feet above the crown of drooping leaves. These thousands of flowering grass trees made one of the most peculiar sights that I remember. The very profuse flowering was attributed to the fact that the ground had been burned over the previous season. It was also on these burnt-over areas that there was the greatest profusion of orchids and sundews. In one such place we collected nearly twenty orchids, of which some were very attractive. These included several species of Caladenia, Glossodia, Diuris—especially showy bright yellow flowers—Pterostylis, and several small species which were not identified. Among the Caladenias were several of the curious species known popularly as "spider orchids," on account of the long slender extensions of the sepals. Hibbertia, Wahlenbergia, and several other pretty flowers were common, and one in particular was very handsome. This was an iridaceous plant, *Diplarrhena Moraea*, with large white flowers that suggested an orchid. The flower is somewhat zygomorphic, and there are but two perfect stamens.

One of the numerous rocky promontories was visited. Much of it was covered with a pretty dense growth of *Melaleuca ericifolia*, which bore a profusion of beautiful snow-white flowers, reminding me somewhat of some of the Californian species of Ceanothus. There were also quite extensive pure stands of *Casuarina* sp. forming small trees.

The exposed point of the peninsula was quite destitute of shrubs of any size, and the nearly flat summit was covered with the prevailing sandy soil in which scattered low shrubs and the usual herbaceous plants were growing.

The most extensive collecting trip was across the divide of the mountain backbone of the promontory. The trail is a pretty good one, and we made it on horseback. The first part of the trail rose steeply over bare rocks and flats of poor sandy soil, covered with dwarf Casuarinas and various low shrubs and herbs. There were some very pretty heaths, and a fine orchid. Thelymitra, with beautiful sky-blue flowers. After climbing for some time at about 1,000 feet elevation, the trail led through the forest, which grew moister and more luxuriant as we neared the summit of the pass.

Along the side of the trail were a good many pretty flowers, perhaps the finest being two bright scarlet heaths, *Epacris* spp. Victoria is expecially rich in these beautiful plants, which seem to prefer the cooler and moister conditions of eastern Victoria. Yellow Hibbertias and pink Tetratheca and Bauera were among the most abundant of these flowers along the trail.

As soon as the divide was passed the effects of the greater moisture on the windward side were apparent, and, as the trail descended, the increasing abundance of mosses and ferns gave evidence of the increasing moisture. Tree ferns began to be abundant in the gullies, and fine specimens of *Todea barbara* were seen. The banks and the rocky beds of the streams showed a rich growth of mosses and liverworts, among them the fine moss, *Dawsonia superba*, collected long ago at the Black Spur, and the beautiful liverwort *Hymenophytum* (*Umbraculum*) *flabellatum*.

There was not time to go to the bottom of the trail, where there are to be seen *Eucalyptus globulus* and the evergreen beech. *Nothofagus Cunninghamii*. Both of these are common Tasmanian species.

At the entrance to the park there are extensive sand dunes, which have been planted with "Marram grass" (*Ammophila arundinacea*), which seems to be very efficacious in holding the shifting sand.

I was unable to visit the Cape Oltway district in southwestern Victoria,. where there are extensive forests of blue gum and beech (*Nothofagus Cunninghamii*).

## South Australia

A large part of South Australia is desert, and the flora is less rich than that of the neighboring states, Victoria and West Australia. As my time was limited, I was able to obtain only the most superficial acquaintance with the flora of this state. South Australia lacks high mountains, the Mount Lofty range near Adelaide scarcely exceeding 3,000 feet altitude,

and this region receives a fair amount of rain; but out of a total area of 380,070 square miles in the state, over 300,000 receive less than ten inches of rain annually.

The region about Adelaide, the principal city, seems to be a fertile one, and when I saw it, after the abundant rains of last year, the young crops of grain and hay and the flowering orchards in the Mount Lofty district gave promise of an excellent harvest. There was the typical Eucalyptus forest, with Acacias and Casuarinas as undergrowth, and in some districts stands of the cypress-like *Callitris* sp.

Along the railway line were in many places masses of the European gorse and broom, which seemed to be throughly naturalized, and, as they were in full flower, they made a brilliant show. In this region, as elsewhere in Australia, a number of South African plants have become naturalized and in some cases are troublesome weeds. The "Cape weed," *Cryptostemma Calendulacea*, covered several of the fields with a solid carpet of pale yellow flowers, and the bright yellow *Oxalis cernua* was extremely abundant in many places. Sometimes in low ground the common calla lily could be seen, and several of the showy Iridaceae of South Africa, Sparaxis, Watsonia, and Homeria, were seen apparently quite naturalized. The latter is said to be poisonous and is regarded as a pernicious weed. At one place, not far from Adelaide, the cardoon (*Cynara Cardunculus*) was very abundant, but it was not noted elsewhere.

Adelaide is most attractively laid out, with a fine park along the river which traverses the town, and a good botanical garden. At the time of my visit, in September, the flowering deciduous trees and shrubs were at their best. Lilacs were in full bloom, and especially beautiful were the double flowered peaches—pink, white, or crimson, which were freely planted in the park and botanical gardens. Unusually large individuals of the Judas tree were also seen.

Of evergreen trees I noted several fine specimens of the redwood and the Californian bay tree (Umbellularia) and a gigantic specimen of the European *Arbutus unedo*. A remarkably fine specimen of *Araucaria imbricata* was a feature of the garden. I was much interested in an extensive collection of South African Iridaceae: Ixia, Sparaxis, Tritonia, and Freesia. The Cape bulbs, as might be expected, do remarkably well in Australia and Northern New Zealand. In the United States, except in the warmer parts of the Pacific Coast, these beautiful plants must be grown under glass.

## Western Australia

Western Australia, from the scenic standpoint, is much inferior to the eastern coastal regions of Australia, as it is largely an extremely arid region and the mountains are low and not at all striking in appearance. The extreme southwest corner, however, has a fairly abundant rainfall, and the coastal portions of this area support a heavy growth of giant eucalypts.

The northern coast, with a tropical climate, has in some portions good summer rains; but for the most part Western Australia is a desert with an annual rainfall of less than ten inches.

Nevertheless, Western Australia is in some respects the most interesting region to the botanist in Australia, as it is here that the autochthonous Australian flora is seen at its best. Nearly four thousand species have been described, and of these a large majority are confined to Western Australia and include many of the most beautiful and peculiar of the Australian plants.

Diels[2] has written a very complete account of the flora of Western Australia, and in the introduction to this has given an excellent description of the most important botanical regions of Australia, with the more characteristic plants of each region.

West Australia is very old geologically, and it is believed that it was here that most of the peculiar Australian types originated. There is evidence that it was formerly separated from the eastern part of the continent, and from it, after the union of the two regions, the autochthonous plants migrated east and north.

Traveling westward by the transcontinental railway, which runs from Port Augusta, in South Australia, to Kalgoorlie, one obtains a good idea of the character of the country comprising much of the dry interior regions of Australia. The country was quite different in appearance from what I had anticipated. I had pictured the "desert" of the interior as a sandy waste, quite destitute of vegetation; but no such areas were seen, although they do occur in many parts of the central plains of Australia.

Along the route of the trancontinental there is, for the most part, no lack of small trees and shrubs. One region known as the "Nullarbor Plains" has only low bushes of salt bush (Kochia, Atriplex) and similar shrubs, much like the sage brush of the Nevada desert; but except in this region there were many trees, sometimes almost abundant enough to be called a forest; and among these were many shrubs of varying size, with bunch grasses and various low-growing plants between.

As usual, the predominant trees were Eucalyptus, and there were also shrubby species, known locally as "Mallee" (*E. oleosa, E. uncinata*). Casuarinas were also abundant, and Acacias were perhaps the commonest of the shrubs. As the latter were in full bloom, they made brilliant masses of gold in the dull gray-greens of the general vegetation. Other characteristic shrubs of this region are sandalwood (*Santalum cygnorum*), *Myoporum* sp., and "Quandong" (*Fusanus acuminatus*).

In many places, grass was sufficiently abundant to indicate excellent grazing country, if only artesian water were available, as the country is quite destitute of any natural streams or springs.

Aside from the Acacias and a few other shrubs, not many flowers were

[2] Die Vegetation der Erde 7. Leipzig, 1906.

seen. At one place, where the train halted, were some pretty everlastings, and some of the passengers brought back bunches of the gorgeous scarlet "Sturt pea" (*Clianthus Dampieri*), one of the most magnificent of Australian flowers.

The transcontinental terminates at Kalgoorlie, where a tiresome day was spent waiting for the train which was to take us on to Perth. The one-time famous mining centre was now almost dead, and the dreary country about offered very little to tempt the botanist, as the barren, sandy waste outside the town showed only scattered clumps of dwarf Eucalyptus scrub, and a few not particularly interesting flowers.

The train for Perth left in the evening, and the next morning we saw something of the wonderful floral display for which Western Australia is famous. All the way to Perth the railway ran through a veritable garden of flowers, including some of the most interesting and beautiful of the West Australian species. Cycads (*Macrozamia Fraseri*) and big grass trees (*Xanthorrhoea* sp.) were very abundant in many places, and here and there the ground was carpeted with solid masses of beautiful pink and white everlastings. Most beautiful of all were clumps of *Leschenaultia formosa*, a member of the Goodeniacae, of a blue so pure and intense as to excel anything I have ever seen—a sight never to be forgotten.

Bright yellow Hibbertias, blue Dampiera, scarlet, yellow, and purple Papilionaceae, pink Boronias, and many others which could not be identified mingled in this gorgeous show, and for the first time I saw one of the most extraordinary of the West Australian flowers, the so-called "Kangaroo paws" (Anigozanthos)—red, yellow, scarlet and green, or pure green. It was a most promising introduction to the floral wonders of this favored region.

Perth, the principal town of the western state, is an attractive city of moderate size, which is an excellent place to see the flora of the coastal region. While there is no formal botanical garden, the city, with great wisdom, has reserved as a park, and left practically untouched, a tract of considerable size along the water front, in which the plants are protected and where one can see most of the beautiful flowers which abound in this region.

The park extends along the bluffs of the river bank, and is an open forest in which the most important tree is the red gum (*Eucalyptus calophylla*), a very handsome and distinct species. An avenue of the splendid scarlet gum (*E. ficifolia*) has been planted, but I was too early to see it in flower. Many Casuarinas of fair size grow among the gums, and two Banksias, one of which, *B. grandis*, was a most striking object. The coarsely serrate leaves are said to be the largest leaves borne by any West Australian tree, and the immense cylindrical yellow inflorescences are extremely conspicuous.

The gritty, sandy soil was covered with a mass of varied vegetation.

Mingled with coarse grasses and sedges was a bewildering variety of low flowering shrubs and bushes. Many of these genera had also eastern species —*e.g.*, Boronia, Dampiera, Hibbertia, Patersonia, Tetratheca, and others; but many belonged to genera mainly developed in Western Australia and quite different from any seen before. The genus Candollea (Stylidium), while occurring in Eastern Australia, is particularly developed in Western Australia, where it includes a great many species, some dainty little plants a few inches high, with delicate pink and white flowers, forming dense patches; others taller and more robust, solitary or few together. The column formed of the stamens and pistil is bent backward and when touched springs out with a jerk, but gradually returns to its original position. They are popularly known as "trigger plants."

The genus Drosera is extraordinarily developed in Western Australia. Some of these species form tiny rosettes, close to the ground, while others have slender half-climbing stems four or five feet long, with flowers almost an inch in diameter.

Small everlastings (Helichrysum?) made solid patches of white in the sandy soil, and another very conspicuous plant was *Ricinocarpus* sp., one of the Euphorbiaceae—covered with masses of snow-white tubular flowers.

The pretty blue and lavender Patersonias were common, the sole representatives of the Iris family; but the Liliaceae were abundant, comprising some very pretty species of Dianella, Thysonotis, and Burchardia. The genus Thysonotis, of which there are a number of species in West Australia, has beautiful fringed petals, usually blue or purple in color. Burchardia has umbels of white flowers, not unlike some species of Allium, or the Californian Brodiaea.

A conspicuous and abundant plant was *Anigozanthos Manglesii* or "Kangaroo paws," a most extraordinary and bizarre flower. The scapes, two or three feet high, have closely set, two-ranked flowers. The tubular flowers are split open and flattened, so that the flower presents a fancied resemblance to the paw of an animal, the six short perianth segments representing the toes. As the outer surface of the perianth has a velvety surface, the comparison is still more striking; but the color is perhaps the most remarkable feature of this species, the perianth tube being of an intense verdigris green, while the inferior ovary is blood-red.

Ground orchids are very common and comprise some very beautiful species. As elsewhere in Australia, the genus Caladenia is the commonest. A fine yellow species (*C. Falda*) was particularly abundant, and when growing in quantity suggested beds of yellow Erythronium. Another species, *C. Patersonii*, is known locally as "spider orchid," as the sepals are drawn out into long, slender filaments. A third species, *C. gemmata*, was a deep blue, and extremely handsome. Other genera, Thelymitra, Glossodia, and Diuris, were represented by several species. Some of the Thelymitras, with racemes of fine azure-blue flowers, are among the handsomest of the orchids.

Grass trees (*Xanthorrhoea* sp.) were abundant, as in other parts of Australia, and Leptospermum, Melaleuca, and the peculiarly West Australian Verticordia, were the most common Myrtaceae aside from Eucalyptus. Some of the species of Verticordia, with delicate pink, finely fringed petals, were particularly noteworthy.

The only gymnosperm was a very abundant cycad—"Zamia palm" in the vernacular (*Macrozamia Fraseri*). This cycad is often responsible for the poisoning of animals which eat the young foliage, especially in dry seasons, or after a fire.

The hills about Perth offer many attractions to the botanist, as they abound in the beautiful flowers for which the whole region is famed. In September and October, the Australian spring, the magnificent Western Australian flora may be seen here in all its glory.

Western Australia is the home of many species of Eucalyptus, including the important timber trees jarrah (*E. marginata*) and karri (*E. diversicolor*). The former has a pretty wide range, usually growing in poor soil. It has a very characteristic habit, the stiff, ascending branches presenting a very different appearance from that of most of the gums. The karri is confined to a much more restricted area in the southwestern corner of the state, in a region of abundant rainfall and good soil. It is the giant among the western gums, and is said to attain a height of three hundred feet. It was a pitiful sight to see the gaunt skeletons of these splendid trees killed by ring-barking, to provide wretched grazing for a few sheep and cattle.

The most beautiful of the showy-flowered gums belong to Western Australia, most of the species having a very limited range. The best known of these, the splendid scarlet-flowered gum (*E. ficifolia*), is known only from a single locality about seventy miles from Albany. Another striking species is *E. macrocarpa*, a shrub of moderate size, whose stems and broad leaves are thickly covered with a white bloom, and whose solitary flowers, as big as a hollyhock, form big pompons of scarlet stamens. This species has broad, horizontal leaves like the juvenile foliage of the common blue gum, probably a permanent retention of the primitive leaf form.

A most interesting visit was made to Albany, on the South Coast. I had the good fortune to have as my traveling companion and guide Mr. C. E. Lane-Poole, Conservator of Forests. I am under great obligations to Mr. Lane-Poole, as well as other government officers, to whom I am indebted for many courtesies which greatly facilitated my work. Professor Sir Edgeworth David of the University of Sydney joined us, and the two days spent in the Albany district were among the most delightful and profitable of my Australian experiences.

While many species noted about Perth were found here, a great many were peculiar to the Albany district. The number of species within a small area was amazing, and it is probable that nowhere in the world could a greater number of species be found within an equal area.

The first day I was driven over a large area in the neighborhood of Albany, and the number of species met with was astonishing. At every stop new things were found, until one was fairly bewildered at the number of novelties. This was largely due to the remarkable number of species in such genera as Drosera, Stylidium, Hibbertia, and the innumerable Papilionaceae.

The region which provided this remarkable variety of interesting and beautiful plants was largely a sandy, moist, peaty moorland. This moorland was quite bare of trees or large shrubs in some places, but often there were groves of Eucalyptus, Casuarina, Banksia, and other small trees, as well as a dense scrub of species of Leptospermum Melaleuca, Leucopogon, and various Leguminosae and Proteaceae. One of the most conspicuous of the latter was a Banksia, with brilliant scarlet flowers. Another remarkably showy shrub was a Callistemon, with huge scarlet bottle-brush inflorescences.

Among the hundreds of showy flowers it would be hard to decide which were the most characteristic. The Droseras were very abundant and of many species. Of the forty-five species found in Western Australia, probably the greater number occur in the Albany district. I failed to find the peculiar Australian pitcher plant (Cephalotus), which is known only from this region. The species of Stylidium were very numerous and varied, and one may guess that a large part of the 84 West Australian species are found near Albany. Various species of Goodenia, Dampiera, and Leschenaultia represented the characteristic Australian family Goodeniacae, and most of the genera already referred to as occurring near Perth were abundant about Albany, but generally represented by different species. Thus the green and scarlet "Kangaroo paws" of the Perth region was replaced by red and yellow, or yellow and green species. Boronia and Tetratheca are especially abundant in West Australia and include some very attractive species.

As elsewhere in Australia, Leguminosae are very much in evidence. Acacias in great variety abound, and are usually known as "wattle." The Papilionaceae are everywhere extremely abundant and comprise a great number of showy species. The colors are often extremely brilliant and the flowers are produced in great profusion. Many genera—Brachyzema, Chorizema, Gastrolobium, Jacksonia—are either entirely West Australian or predominantly so.

The Umbelliferae are fairly well represented and include some very peculiar types. Perhaps the most striking genus is Actinotus, one species of which, *A. Helianthi* of New South Wales, is known as "flannel flower," the inflorescence being very suggestive of the Swiss Edelweiss. In Western Australia the showy "southern cross," *A. rotundifolia*, is common. Space forbids a further enumeration of the Albany flora, which I think is the most varied that I have ever seen.

In company with Mr. Lane-Poole and Professor David, I visited Denmark, some forty miles from Albany, and once an important lumbering

district; but now most of the fine karri timber has been cut or killed, and only a few remnants of the great forest are left in this district. This region has a rainfall of approximately 60 inches, and the Western Australian forest reached its maximum development here.

Our means of transport was a motor railway "trolley," a small open car that was just sufficient to hold the three of us in addition to the driver. It was a decidedly novel experience and rather alarming at first, but we soon grew used to the motion and could enjoy the many interesting plants growing along the route. The track was often bordered with shrubs covered with beautiful flowers of all colors, and in many places were veritable forests of grass trees in full flower. In addition to the Xanthorrhoeas, there were also many specimens of the peculiarly West Australian Kingia, much like Xanthorrhoea in habit, but having small globular inflorescences on short stalks, looking like drum-sticks. The grass trees reach their greatest development in this region. They often branch, and the tall flower spikes, eight or ten feet high, stand out conspicuously above the crown of slender, drooping leaves.

The scenery in the vicinity of the karri forests is attractive. Owing to the abundant rainfall, there are clear streams of considerable size and rich and varied vegetation. Near the river banks grow the largest of the Banksias, *B. verticillata*, a tree 60 or 70 feet in height, with a trunk two feet thick.

The abundance of Loranthaceae throughout Australia has already been mentioned, but the most extraordinary member of the family is confined to Western Australia. This is *Nuytsia floribunda*, known in some districts as "Christmas tree" as it flowers at Christmas time; from all accounts it must be a most magificent sight. I saw many individuals, but none in flower. It is a small tree, a root parasite, and when in flower is said to be completely covered with a mass of orange-red flowers.

Western Australia is peculiarly interesting to the botanist, as the flora is almost exclusively made up of the strictly Australian types. The Malayan elements, which are so conspicuous in the forests of Queensland and New South Wales, are almost completely absent from Western Australia. The flora is also notably poor in ferns and bryophytes, few of which find a congenial home in this region with its poor, sandy soils and long, hot, dry summers.

Fortunately for the botanist, the region about Albany is not well adapted to agriculture and is likely to remain for a long time a happy hunting ground for the flower lover.

The autochthonous Australian flora reaches its extreme development in Western Australia, and there is an almost complete absence of any forms related to either the Malayan flora of Queensland and New South Wales, or the Fuegian element developed in Tasmania and the higher mountains of Eastern Australia.

A very large majority of the 4,000 species of West Australia are confined to that state, and there are many endemic genera as well as species. A notable feature of the flora is the remarkable number of species within a genus.[3] Drosera has 45 species, Candollea 84, Boronia 44, Goodenia 48, Grevillea 70.

Wallace (Island Life, 2d edition, p. 494) believes that Southwest Australia represents the remnant of an ancient, more extensive, isolated land area within which were developed the ancestors of the present autochthonous Australian flora, and this view seems to have much probability. There is strong evidence, as has already been stated, that in Cretaceous times Western Australia was completely separated from the eastern part of the continent, the western portion of which was probably united with New Guinea. The flora of North Queensland and coastal New South Wales still retains a large Malayan element, the "scrubs" of Queensland being predominantly Malayan in their flora.

It is highly probable that in early Tertiary times, before the Union of East and West Australia, the former region was occupied by a flora of exclusively Malayan character, while in the western continent the ancestors of the modern Myrtiaceae, Proteaceae, and other characteristic Australian types were completely segregated.

It is not unlikely that this western continent, owing to the intrusion of the sea in Cretaceous times, had a more uniform and less arid climate than now prevails in most of West Australia. It may have been like that now found in the extreme southwest, where a great majority of the existing species occur.

With the establishment of the connection between east and west, the existing climatic conditions were developed within the great arid central regions which occupy so much of the present continent.

With the increasing aridity in Western Australia is to be associated the evolution of the predominant xerophytic habit found in most of the typical Australian plants. These xerophytic forms apparently migrated east and north and took possession of most of the eastern territory. At the present time, all that is left of the ancient flora of northeastern Australia is probably the "scrub," confined to the narrow coastal belt of Queensland and New South Wales. This scrub is not continuous, but is surrounded by much larger areas of xerophytic Eucalyptus forest.

A few species of Eucalyptus and the allied genera, Tristania and Angophora, grow in some of the rain forests, and it may be that the few Proteaceae which occur in the rain forests, like *Grevillea robusta*, Macadamia, and Stenocarpus, are descendants of Western immigrants which have become adapted to the changed environment.

If the assumption is correct that the autochthonous Australian vegetation originated in Western Australia, the question then arises as to the origin of the ancestral forms from which this flora descended.

[3] Maiden, *loc. cit.*, pp. 183–190.

The region which shows most similarity to West Australia in its flora is the Cape region of South Africa. There is a similar development of Proteaceae, as well as certain Compositae common to the two, and the true heaths (Ericaceae) of the Cape are very similar in many respects to the Australian Epacridaceae. There are, however, many differences, and any land connections that may have existed must have disappeared at a very remote period. There is clear evidence of a connection of all the southern land masses in the Permian—the so-called "Gondwana Land," but the evidences for later connections are, at present, more or less problematical. However, it seems pretty certain that some connection between South Africa and Australia did exist, perhaps in the Tertiary, and that there is a real relation, although remote, between their floras.

It may be that further investigation will show that in the Tertiary, as was the case in the northern hemisphere, there was a practically uniform flora, occupying a more or less continuous land mass connecting the now widely separated regions of South America, Australia, and South Africa. It is possible that the common elements in the floras of Australia and South Africa are descendants of this ancient flora, which through long isolation have diverged from each other.

The complete isolation of Western Australia has resulted in a remarkable degree of specialization among a relatively small number of original types, with almost no admixture of immigrants subsequent to the severing of the connection of Australia with the land to the south.

## EXPLANATION OF PLATES

PLATE XXI

FIG. 1. Tree ferns, Botanical Gardens, Melbourne.

FIG. 2. *Banksia grandis*, Perth.

FIG. 3. Chaparral formation, National Park, Victoria. The trees are *Casuarina* sp.

FIG. 4. Desert vegetation, transcontinental railway.

FIG. 5. Coastal vegetation, Perth. The largest tree is a red gum (*Eucalyptus calophylla*).

PLATE XXII

West Australian grass trees (*Xanthorrhoea reflexa*). Photograph furnished by Mr. C. E. Lane-Poole.


CAMPBELL: AUSTRALASIAN BOTANICAL NOTES

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# EVOLUTION AND GEOGRAPHICAL DISTRIBUTION OF THE GENUS VERNONIA IN NORTH AMERICA

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The genus Vernonia, with its vast assemblage of over 500 species, ranges through the western hemisphere from Argentina to Manitoba, occupying a region of great climatic variation and habitats of great ecological diversity. In preparing manuscript for the treatment of the genus in the "North American Flora," 123 species have been recognized north of Colombia and Trinidad. Within this number a few stand comparatively isolated from all the others, while many are so closely related in form and structure and so similar in distribution that they must be closely akin genetically. Over 30 species-groups may be distinguished in this way. Within these minor groups evidences of specific evolution correlated with geographic distribution are frequently seen, while most of the groups, considered each as a whole, present strong evidence in favor of their relation to, and probable origin from, each other. It is therefore possible to build up a general scheme of evolution and migration within the genus in which the two lines of evidence, structural and geographic, complement and support each other.

There can be little doubt that the ancestral home of the genus, as far as North American species are concerned, is tropical South America. This is shown by the presence there of a large number of species of greater structural diversity than exist on the North American continent, and also by the fact that many South American species are of a structure which clearly indicates their primitive nature.

Within the genus as a whole, the more fundamental structural differences, which have been used in the division of Vernonia into its many sections and subsections, relate chiefly to the structure of the achenes, the pappus, and the involucral scales. Nothing can now be said concerning the possible evolution of these groups. Of those distinguished by Bentham and Hooker in "Genera Plantarum" and accepted by Hofman in "Die natürlichen Pflanzenfamilien," four have reached North America.

1. The section Stengelia, of the East Indies, with veiny, foliaceous involucral scales, is represented by a single species, *V. anthelmintica* (L.) Willd., sparingly introduced into a few islands of the West Indies. Certain Mexican species which bear a superficial resemblance to this section appear to belong rather to the section Lepidaploa, and their similarity to Stengelia is better explained by convergent evolution.

2. The section Tephrodes, of the paleotropical region, with terete achenes, is represented by a single species, *V. cinerea* (L.) Less., widely introduced

as a weed in tropical America and recently reported from extreme southern Florida. Its further migration through the agencies of commerce is to be expected.

3. The section Stenocephalum, with coriaceous, spine-tipped involucral scales, represented by several species of tropical South America, has a single little-known and recently discovered species, *V. jucunda* Gleason, in the mountains of southern Mexico. If we assume from the negative evidence at hand that the section is actually absent from Central America, we may infer an early migration of the section northward, followed by extinction in Central America and the isolation of the single species in Chiapas.

4. The section Lepidaploa, with membranous involucral scales and ribbed achenes, includes 120 species of North America and many more in South America.

Within this large section there is still well-marked evidence of evolutionary development in structure, illustrated most plainly by the character of the inflorescence. One type of inflorescence may be assumed *a priori* as the most primitive, and from it by successive small changes all the other types may be derived. Since the center of evolution and migration for the genus is considered to be tropical South America, where this primitive type is largely developed, and since the succeeding stages in the modification of the inflorescence occur progressively farther to the north, structure and distribution complement each other, and it may be assumed with little hesitation that migration and structural evolution have proceeded simultaneously; that the tropical species, while not necessarily the oldest in time, are at least the most primitive in structure, and that the outlying species of the temperate part of North America are both young in age and advanced in evolution. It can not now be stated whether there is a similar correlation between structure and distribution among the species of South America.

In the section Lepidaploa, the inflorescence is either a scorpioid cyme or some other type of cluster obviously derived from it by certain apparent modifications in the original structure. In such an inflorescence each head is morphologically terminal: a lateral branch, arising at the first node below the involucre, terminates in a second head and bears another lateral branch which behaves in the same way. There is thus produced a more or less elongate sympodial axis, morphologically indeterminate in its development, and with its series of truly terminal heads apparently lateral and secund along it. Since the successive lateral branches arise from nodes, which are normally marked by leaves, it may be assumed at once that the leafy scorpioid cyme is the primitive inflorescence, while those species in which the bracteal leaves are suppressed stand relatively higher in the scale of evolution.

Each segment of such an inflorescence then consists of a basal internode with a leaf at its summit and a head beyond it. The structure of the bracteal leaves varies greatly, but in general they maintain the form and pu-

bescence of the cauline leaves and differ from them chiefly in size. The head is pediceled if separated from the bract by an obvious internode, and the inflorescence is then a scorpioid raceme. If this internode is reduced, the head is sessile and the cluster becomes a scorpioid spike. The clusters may be straight or flexuous, long or short, crowded or loose, with heads ranging from 2 to 25 in number. As a result of the straightening of the sympodial axis, the heads appear lateral and are usually placed about 90° around the axis from the bracteal leaf.

The leafy scorpioid cyme is found in 57 species, ranging throughout the West Indies and on the continent extending north into southern Mexico. Species with leafless cymes, 63 in number, occur commonly on the continent from Panama to New England. From this region four have crossed the narrow gulf east of Yucatan and entered Cuba; one has reached the Bahamas from Florida; one is endemic to St. Vincent, and another reaches Trinidad and the neighboring islands. In general, the distribution of the latter group is continental, of the former Antillean.

Perhaps the simplest type of the primitive leafy inflorescence is found in the species-group Graciles, in which the cymes are stemlike and quite undifferentiated from the truly vegetative portion of the stem, with bracteal leaves closely resembling the cauline in size and shape. Species of this group are almost entirely South American, ranging, according to Ekman, from Colombia to eastern Brazil. One species only, *V. gracilis* H. B. K. var. *tomentosa* Ekman, of Bequia, occurs in our range.

A second group, with almost equally simple inflorescence, composed of long, irregular, branching cymes, with long internodes and leaflike bracts, is the Argyropappae, of tropical South America, Central America, and southern Mexico. The South American origin of the group may be assumed. From there, *V. remotiflora* Rich. has been introduced into St. Thomas; *V. acilepis* Benth. is endemic to Costa Rica, and *V. argyropappa* Buek extends from Peru to Mexico. Two offshoots of the latter have arisen in Mexico, *V. hirsutivena* Gleason in Yucatan and *V. ctenophora* Gleason in Campeche, differing in minor structural details.

It will be observed that these two groups, simplest in structure, are distributed primarily in South America and that only a part of their species reach North America, although among these are three endemic species and one endemic variety.

A third group of similar primitive structure as to inflorescence is the Schiedeanae, of Central America and southern Mexico. While its members differ sharply from the preceding group in their large heads, the peculiar development and specialization of the involucral scales, and the absence of foliar resin dots, they retain the simple cymes and broad, heavy leaves, and may possibly be derived from it. *V. verrucosa* Klatt, with narrow acuminate scales, appears to be the simplest and is endemic to Costa Rica. *V. Seemanniana* Steetz follows in Costa Rica, with broad, obtuse scales,

and the greatest modification is found in *V. Schiedeana* Less., ranging from Honduras to Vera Cruz, with involucral scales broadly dilated at the tip. The progressive specialization in structure, correlated with increasing distance to the north, is here clearly shown.

There now follow seven species-groups with 33 species, all West Indian, all clearly related, and all exhibiting a remarkable correlation between structure and distribution.

The most primitive of these, from which the other six are directly or indirectly derived, is the Arborescentes, ranging from the Windward Islands to Jamaica. The wide range and primitive structure probably indicate an early arrival in the region. The most primitive species, *V. icosantha* DC., has stems bearing leaves of normal size to the apex and terminating in a single sessile head. At the base of this head the two primary cymes arise; they are straight, elongate, with prominent internodes, sparingly branched or simple, and bear numerous heads. The chief distinction in the inflorescence between it and the Graciles is the regular presence of paired primary cymes. In *V. sericea* L.C.Rich., of the Virgin Islands and Porto Rico, the cymes are shorter and more frequently branched. *V. borinquensis* Urban, of Porto Rico, has exceedingly flexuous, many-headed, freely branched cymes, the branches invariably arising at the base of a head. *V. arborescens* (L.) Sw., of Jamaica, has numerous frequently congested cymes and reduced bracteal leaves. *V. permollis* Gleason, of Jamaica, completes the group, with congested cymes and an unusual development of foliar pubescence. The general tendency of the group is toward the production of cyme-branches and supernumerary cymes, making a congested inflorescence in which the bracteal leaves are reduced.

The Longifoliae, a group of three species, is related through *V. longifolia* Pers., of the Lesser Antilles, to *V. icosantha*. Superficially the two species are much alike, but the inflorescence in the former shows a distinct difference. The primary cymes are short, compact, divergently spreading at an angle of 60–90°, crowded, bearing only 2–5 heads on short internodes with bracteal leaves considerably smaller than the heads. Secondary cymes arise just below the primary in the upper leaf axils. They are essentially leafless for the first 2–5 cm., and then bear toward the summit either the usual crowded heads or a terminal head subtended by two short cymes. This whole inflorescence terminates completely the growth of this portion of the stem, but during the next vegetative season new branches appear from the next lower axils in order, grow out at a divergent angle, soon surpass the old cymes of the previous season, and at the next blooming season bear their cymes in turn. The plant has therefore a method of continuing its vegetative growth beyond one season, and as a result reaches a considerably larger size. *V. Shaferi* Gleason, of Montserrat, is closely similar, and represents an island endemic. *V. albicaulis* Pers., of the Virgin Islands and Porto Rico, preserves the same inflorescence but differs in its obtuse or broadly rounded leaves.

It is a comparatively short distance across open water to the north of Porto Rico, the home of *V. albicaulis*, to the southern islands of the Bahamas. In these southernmost islands occurs *V. bahamensis* Griseb., the most primitive member of the species-group Bahamenses. The fundamental difference between this group and the Longifoliae is again found in the inflorescence. Here the cymes, after the flowering period, continue their elongation into the vegetative shoots of the next season. Not every cyme necessarily elongates, but there are regularly 2–4 such branches at the apex of each year's growth. Toward their base, paired scars mark the location of former heads and bracteal leaves, while above them scars in spiral arrangement indicate the former position of foliage leaves. All these Bahaman species are therefore bushy, widely spreading, freely branched shrubs. It is particularly worthy of note that they all have broad obtuse to retuse leaves; that *V. bahamensis*, the species most nearly resembling *V. albicaulis* in leaf form, is the species of the southernmost islands, and that the particular specimen in herbaria which most closely approximates the leaves of the Porto Rican plant in size was collected on South Caicos Island, almost the extreme southeastern island of the group. *V. arbuscula* Less. and *V. obcordata* Gleason occur farther to the northwest in the Bahamas. *V. complicata* Griseb., of eastern Cuba, differs only in minor characters. It is difficult to imagine a more striking instance of correlation between structure and distribution than is presented by this group in its relation to the Longifoliae.

The last three species-groups illustrate the following course of development in the inflorescence:

1. The cyme is a special branch with reduced bracteal leaves and elongate axis.
2. The cyme and leafy branches differ merely in position, and the inflorescence is compact.
3. The cyme becomes the leafy branch at the conclusion of the blooming season, and the inflorescence is compact and reduced.

The fourth species-group of the seven, the Racemosae, includes five species of Hispaniola and Cuba. They are probably derived from *V. sericea* of the Arborescentes, which is located near by in Porto Rico; *V. racemosa* Delp. was considered by Ekman a variety of *V. sericea*, and, like it, most of the species have leaves pubescent on the lower surface. In this group the two upper primary cymes are short, with only 2–5 heads. Below them, every leaf axil for a considerable distance down the stem produces similar short lateral cymes. The whole makes an elongate subcylindric inflorescence, quite different from the broad, spreading type of the preceding groups. Secondary vegetative branches apparently do not exist. Within the group, evolution is seen in the progressive reduction of the leaf surface, of the cymes, and of the number of flowers in the head. While *V. racemosa* of Hispaniola has lanceolate leaves, and cymes of 2–5 many-flowered heads,

the next three, one of Hispaniola and two of Cuba, have progressively narrower and more revolute leaves and smaller and fewer heads. The group culminates in a fifth species, *V. corallophila* Gleason of Cuba, with linear leaves revolute to the midvein, 11-flowered heads, and 1-headed cymes which appear as single axillary heads.

A fifth species-group, the Gnaphaliifoliae, also appears to be derived from *V. sericea* or some species similar to it. There is the usual terminal head, subtended by two primary cymes, and numerous other cymes arise from the upper axils. They are usually flexuous, spreading or ascending, and only occasionally branched. Such structures point unmistakably to an origin within the Arborescentes, with which they share many structural features and from which they are indeed rather weakly separated. The three species are all Cuban. In this group axillary branches do not continue the vegetative growth, but the whole herbaceous stem dies and is replaced by new growth from the perennial base.

All groups heretofore described in this general series have acute or acuminate involucral scales. The sixth, the Acuminatae of Jamaica, have obtuse scales, and also differ from the Arborescentes in their resinous-dotted, non-papillose leaves and in their flattened and twisted pappus bristles. Nevertheless, the simplest species of this group bears a general resemblance to *V. arborescens;* has been placed adjacent to that species by Ekman, and may have been derived from it. I fail completely to see any resemblance between this group and the Fruticosae, as has been claimed by Ekman. *V. acuminata* Less. is the common species of lower elevations. *V. pluvialis* Gleason, the high-mountain derivative, presents an inflorescence of short, much congested cymes, aggregated in subcapitate clusters.

The seventh and last group of this series, the Fruticosae, includes one species of Hispaniola and eight of Cuba, particularly of the mountains of the eastern part. Many of these are poorly known, some by a single collection, and the number of species may easily be subject to increase or decrease as further collections are accumulated for study. From the inflorescence standpoint, they exhibit the simplest scorpioid cymes to be found in the West Indies. They are mostly straggling vinelike plants with indeterminate growth. At some distance above the base the main axis ends in a terminal head, while immediately beneath it a lateral branch, diverging at a small angle, continues the sympodial axis and bears heads in the same way. The heads are separated by internodes about equal to those of the sterile section of the stem in length, they are subtended by bracteal leaves which in almost all species are virtually indistinguishable from the cauline in size and shape, and furthermore the cyme axis is frequently prolonged after flowering into a leafy, sterile stem. In their leaf habit and papillose pubescence they approach *V. gnaphaliifolia;* in other features they have no near relatives in the West Indies and apparently none in South America. Nevertheless, they offer no new structural features to separate them from

the preceding species-groups. In the lack of sufficient material, the evolution within the group can not now be discussed.

It is not necessary to presume that only one ancestral stock of Vernonia migrated into the West Indies. The seven species-groups just described, constituting probably one evolutionary stock, have spread over the whole region and developed into many species. Other stocks may also have immigrated from South America, been isolated in certain islands, and developed endemic species. Certainly two species-groups now exist whose relations can not be explained, and which should probably be considered as entirely distinct evolutionary lines. These are the Buxifoliae and the Sagraeanae.

The Buxifoliae include three species of the mountains of Hispaniola. They are characterized by glabrous achenes, heads in subcapitate clusters, and an unusually large number of involucral scales, arranged in a beautifully spiral imbrication.

The Sagraeanae include ten species, nine in Cuba and one in Hispaniola, with an outlying variety in Dominica, characterized by large glabrous achenes and usually by large many-flowered heads. Ekman would relate the group to the Bolivian *V. robusta* Rusby, which differs in achenes, hispid in the furrows, and in the number of setae of the pappus, about 25, instead of 40–70; also to the Bolivian *V. obtusata* Less. (*V. subacuminata* Hieron.) which has densely hirsute achenes. There is a superficial resemblance to these Bolivian plants in their heavy, rugose, reticulately veined leaves, and to *V. robusta* also in their large heads. On the ground that specialized involucral scales, few-flowered heads, and rigid, coriaceous, or tomentose leaves are characters which indicate an evolutionary advance, *V. Sagraeana* DC. and *V. viminalis* Gleason may be regarded as the most primitive species, and *V. Wrightii* Sch.-Bip. and *V. purpurata* Gleason as the most advanced.

We have now disposed of all leafy-bracted scorpioid species of North America except two, *V. yunquensis* Gleason and *V. segregata* Gleason. These Cuban species are poorly known and the former is represented in herbaria only by the type specimen. While each of them exhibits certain points of resemblance to other West Indian species, it is not possible to draw any conclusions as to their relationships.

The general affinities of the 57 species of the leafy-bracted groups may be summarized by the diagram (fig. 1), from which it may be seen that without exception the more advanced groups lie progressively farther from South America, that no group is common to the West Indies and the continent of North America, except as introduced, and that, with very few exceptions, the more advanced species of each group also lie farther away from the center of origin, either in horizontal or in altitudinal distance.

The 63 species in which the bracteal leaves are suppressed show certain fundamental differences among themselves in the structure of the inflorescence, as a result of which five well-marked evolutionary stages may be

distinguished. In the first of these the cymose structure is obvious, each cyme is more or less elongate with secund heads, and branches occur at such intervals that the scorpioid structure is not obscured. In the second, secondary branches are developed at the bases of a great many heads, so that three successive nodes without branches rarely occur. The result is a large branching cluster which bears little superficial resemblance to the

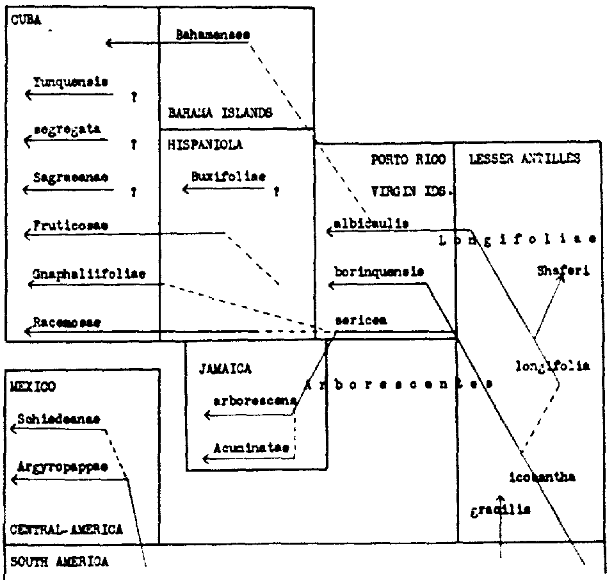


FIG. 1. Migration and evolution of the leafy-bracted Vernoniae of North America. Solid lines show distribution by their location, migration by the direction of the arrow. Dotted lines show probable connection by evolution between species-groups.

simple scorpioid cyme, although undoubtedly derived from it. In the third, the heads are suppressed at those nodes where secondary branches are developed. Since these appear at virtually every node, only terminal heads are produced on the cymes, and the whole cluster appears to be dichotomously branched. The fourth stage represents a much greater step forward. Here the basal internodes of the inflorescence are much shortened or almost suppressed, while the number of heads is greatly reduced. Since the terminal internodes retain a normal length, the whole inflorescence appears subumbellate. While in the first three stages new vegetative branches may arise from below the inflorescence, so that the stem may live several years and reach a large size, in the fourth type, as well as in the fifth, the appearance of the inflorescence prevents further growth

of the stem, and new vegetative parts appear only from the perennial base of the plant. In the fifth and last stage, only a few terminal and subterminal heads retain the umbellate arrangement, while from the upper stem axils similar clusters arise, producing a broad, flat-topped or hemispheric cluster with all the heads peduncled. These five stages are shown diagrammatically in figure 2.

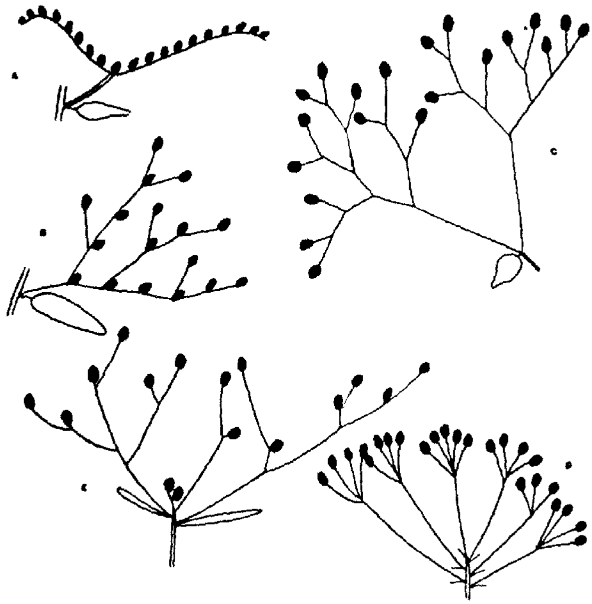

FIG. 2. Modifications of the inflorescence in the bractless Vernoniae of North America *A*. Stage 1, *Vernonia scorpioides*, lateral branch with two terminal cymes. *B*. Stage 2, *canescens*, lateral portion of the inflorescence. *C*. Stage 3, *havanensis*, portion of a terminal inflorescence. *D*. Stage 4, *Karwinskiana*, terminal inflorescence, with a few primary branches omitted. *E*. Stage 5, *texana*, complete terminal inflorescence. All figures diagrammatic as to position of branches or cymes, but accurate as to character of branching and proportion.

Other evidences of evolution appear within the last two stages, leading to the segregation of several species-groups.

The five stages are again well correlated with their geographical distribution. The first occurs in South America and is represented in our region by a single species of St. Vincent, *V. pallescens* Gleason, and by two which extend across the Isthmus of Panama into southern Central America, *V. scorpioides* (Lam.) Pers. and *V. brachiata* Benth. The second includes

several species of northwestern South America, some of which extend into Central America also, and several others ranging as far north as southern Mexico. Species of the third stage have crossed the narrow channel from Yucatan and are now limited to western Cuba; the fourth is confined to Mexico, and the fifth has four species in northern Mexico and 31 in the United States and the Bahamas.

The species-group Stellares, representing the second stage, includes the commonest species of the mountains of Colombia, Central America, and southern Mexico. The Colombian species *V. canescens* H.B.K. and *V. mollis* H.B.K. retain the primitive character of acuminate involucral scales; the former also extends north into Mexico, and the latter is doubtfully admitted into the North American flora. Of the remaining five with acute to rounded scales, *V. patens* H.B.K. occurs in both continents, while the others are strictly North American. The most advanced morphologically is *V. morelana* Gleason, which alone does not occur south of Mexico.

The third stage includes the species-group Menthaefoliae, undoubtedly closely related to the Stellares, but now isolated in western Cuba and the Isle of Pines, except for a few specimens from central and eastern Cuba as well, where they may have been recently introduced.

The fourth stage includes three well-marked species-groups, which are nevertheless closely related. The Umbelliformes include 9 species, mostly quite closely related and in some cases separated with difficulty. The simplest species (and the commonest in herbaria) have small heads, with short involucres and seldom more than 15 flowers; the more advanced have larger heads and taller involucres. One of these, *V. Conzattii* Robinson, with its abruptly rounded and mucronate involucral scales, marks a transition to the group Mexicanae, with three species in the higher mountains of southern Mexico. Here the scales are extraordinarily specialized, being 3–8 mm. wide, loosely spreading, at least at the tip, and prominently reticulately veined. The two closely related species of the Alamanianae have also large scales but lack the reticulate venation. The general evolutionary tendency of the series is apparently toward large heads and specialized scales, and this is correlated geographically with an ascent to higher levels in the mountains.

Passing now to the 35 species of the fifth stage of evolution, as shown by the inflorescence, we find the most primitive members in the Texanae, a group of four species, three in northern Mexico and one in Texas. Since the inflorescence has already passed to the paniculate stage, equally characteristic of the other species of the United States, evidence for the primitive character of the group must be sought in other characters. The leaves in all four species are more or less pitted beneath and the outer pappus bristles are poorly differentiated from the inner in width, both of which features occur also in the Umbelliformes. The most important primitive character, however, lies in the involucre, and has not been mentioned before because

it is shared by virtually all the groups hitherto discussed. Here the scales are relatively few in number and poorly imbricated. The inner scales are progressively more exposed than the middle and outer ones, contrasting plainly with the numerous regularly imbricated scales of most other species of the United States. Of the four species in the group, *V. texana* (A. Gray) Small, is best known and occurs in Texas, Louisiana, and Arkansas.

From the area of the Texanae, migration accompanied by specific evolution has proceeded in two directions, northward through the prairie region and eastward along the coastal plain. In each direction one or more of the primitive structures have been lost, until in Michigan and Massachusetts they have disappeared completely.

In northern Texas occurs the group Lindheimerianae of three species, two of which are suspected to be hybrids. *V. Lindheimeriana* Gray & Engelm., which is undoubtedly a good species, retains the primitive involucre and narrow outer bristles of the Texanae, from which it seems to be derived, and differs chiefly in its tomentose leaves and scales.

The Fasciculatae, extending from Texas and New Mexico northward and eastward to Manitoba and Ohio, retain the pitted leaves and present to a still greater degree the narrow, undifferentiated outer bristles of the pappus. They have lost the primitive involucre and have developed long heads with numerous scales imbricated with great regularity. It is noteworthy that the more southern species, as *V. marginata* (Torr.) Raf., still show a tendency toward acumination of the scales, as in Texanae, which character is lost to a large extent in *V. fasciculata* Michx., ranging from Nebraska to Ohio, and completely in *V. corymbosa* Schw., distributed along the Red River of the North in Minnesota, the Dakotas, and Manitoba, where it marks the extreme northern limit of the genus.

The peculiar local species, *V. Lettermanni* Engelm., of Arkansas and adjacent Oklahoma, bears a strong superficial resemblance to *V. fasciculata* and is possibly an evolutionary development from it. It retains the glabrous leaves with pitted lower surfaces and the congested heads with closely imbricated scales, like the latter species, but has broader, well differentiated outer pappus bristles.

The group Interiores takes its name from *V. interior* Small, which is undoubtedly the basic species. Common in central and northern Texas, where it overlaps the range of the ancestral Texanae, it extends north to Nebraska, and thence east to the Mississippi River. The involucre in this species has only partially lost its primitive structure; the outer pappus is narrow but nevertheless plainly differentiated; the leaves are broad, without pits, and characterized by multilocular hairs forming a more or less tomentose pubescence. *V. Baldwini* Torr. is an Ozarkian derivative, with broader outer pappus bristles, and with the acuminate involucral scales recurved at the tip and pubescent on the inner face. The species is probably of recent origin, and specimens from the overlapping ranges of *V. Baldwini*

and *V. interior* are frequently intermediate in character. *V. aborigina* Gleason, known so far only from the original collection in southeastern Oklahoma, appears to be a giant form of *V. Baldwini*. It retains most of the morphological characters of that species, but is much larger in all its dimensions, with about twice as many flowers in each head. *V. missurica* Raf., the last of the group, has the widest distribution of the four, ranging from Texas, where it is not particularly common, north and east to Michigan, and becoming exceedingly abundant in Iowa, Illinois, and Indiana. It is characterized by larger, more compact inflorescence, fully differentiated pappus, and regularly imbricated involucre. Many specimens from the southern part of its range retain the sharply acute, relatively narrow involucral scales of *V. interior*, while those from farther east have fewer, broader, and obtuse or apiculate scales. The species also occurs to a limited extent and with slightly different structure along the Gulf Coast as far east as Alabama.

The origin of two other western species is in doubt. *V. Bolleana* Sch.-Bip., of northwestern Mexico, seems to bear no close relation to any other known species. *V. crinita* Raf., of the Ozarkian region, is characterized by filiform involucral scales, and may represent an extreme development from the Interiores.

The eastward migration along the coastal plain from Texas led to the present development of seventeen species. They are not easily divided into distinct species-groups, a feature possibly indicative of recent immigration and evolution. The most primitive group is the Angustifoliae, ranging from Louisiana east to the Atlantic, thence north to the Carolinas and south into Florida and the Bahamas. The group retains the primitive involucre, narrow leaves, and low stature of the Texanae, and the type species of that group was originally described as a variety of *V. angustifolia* Michx. Some of the species have an inflorescence approaching umbelliform and rather suggestive of *V. liatroides* DC. or other species of northern Mexico. *V. angustifolia* has the widest distribution, almost coextensive with that of the group. The other four, each of restricted distribution and lacking the acuminate scales of the simpler species, seem to represent recent evolutionary developments. Of these, *V. Blodgettii* Small, in southern Florida, marks the re-entrance of the group into the tropics, and leads to the closely related *V. insularis* Gleason of the nearby Bahamas.

The group Pulchellae is obviously closely related to the Angustifoliae, as shown by narrow leaves and general vegetative habit, but differs in the absence of resin glands on the achenes and in the prolongation of the involucral scales into filiform appendages. The three species, *V. pulchella* Small, *V. recurva* Gleason, and *V. scaberrima* Nutt., are all of limited distribution in the coastal plain of Georgia and the Carolinas.

The species-group Glaucae lies generally to the north of the Angustifoliae and has probably been derived from it. Here the heads are larger,

the pappus is tawny or almost white, and the involucral scales are long-acuminate or almost filiform. The leaves are large in proportion to the height of the stem, and the greatest expanse of foliar surface is toward the base of the stem. While this feature is apparent in *V. glauca* (L.) Willd., an Alleghenian species ranging northward to Pennsylvania, it is still further developed in *V. acaulis* (Walt.) Gleason and *V. georgiana* Bartlett, two coastal plain species with distinctly basal leaves.

Two other species with prolonged filiform scales constitute the group Noveboracenses. *V. noveboracensis* (L.) Michx. has attained a wide distribution over the Piedmont region of the eastern states from Mississippi to Massachusetts, occasionally invading the coastal plain also. There it has given rise to a localized species, *V. Harperi* Gleason, characterized by larger heads with more numerous flowers.

*V. gigantea* (Walt.) Britton is closely related to *V. concinna* Gleason, of the Angustifoliae, and like that species is confined to the southeastern portion of the coastal plain.

The last species-group of the southeastern states is the Altissimae. *V. ovalifolia* T. & G. is a variable species of the southeastern coastal plain, and appears to be the most primitive species of the group in their evolution from the Angustifoliae. Although some of its variants approach *V. concinna*, it is generally distinguished by the broader, regularly imbricated involucre and the broad leaves. *V. flaccidifolia* Small is a well-marked species of the southern Appalachian region. *V. altissima* Nutt., the last species of the group, has a wide distribution from Georgia and Alabama north and west to New York and Missouri. Typical forms of the species avoid the coastal plain and are characteristic of the woodlands of the central states, but the variety *laxa* Gleason occurs along the Gulf Coast. In its western extension *V. altissima* comes in contact with several species of the western migration route, and many intermediate forms occur which are probably to be considered as hybrids.

Considering the 35 species with paniculate inflorescence as a whole, we see that the species with primitive involucre invariably lie far to the south or southwest, and that those with the broadest and most obtuse scales, as well as those with the most filiform scales, lie always well to the north or northeast. It is also worthy of note that only seven of the 35 have attained a wide distribution, while the other 28 occupy small or localized ranges. These seven are *V. fasciculata* Michx., *V. interior* Small, *V. missurica* Raf., *V. angustifolia* Michx., *V. glauca* (L.) Willd., *V. noveboracensis* (L.) Michx., and *V. altissima* Nutt., representing six species-groups. This fact alone may indicate the comparatively recent immigration and incomplete evolution of the genus in the northern portion of its range.

The general relations of the 63 species of the genus in which the bracteal leaves are suppressed is exhibited in a diagram (fig. 3).

In the evolution of the genus in North America, no general plan or

tendency is apparent, except in the one feature of inflorescence. In every case it is found that species structurally aberrant from the general type

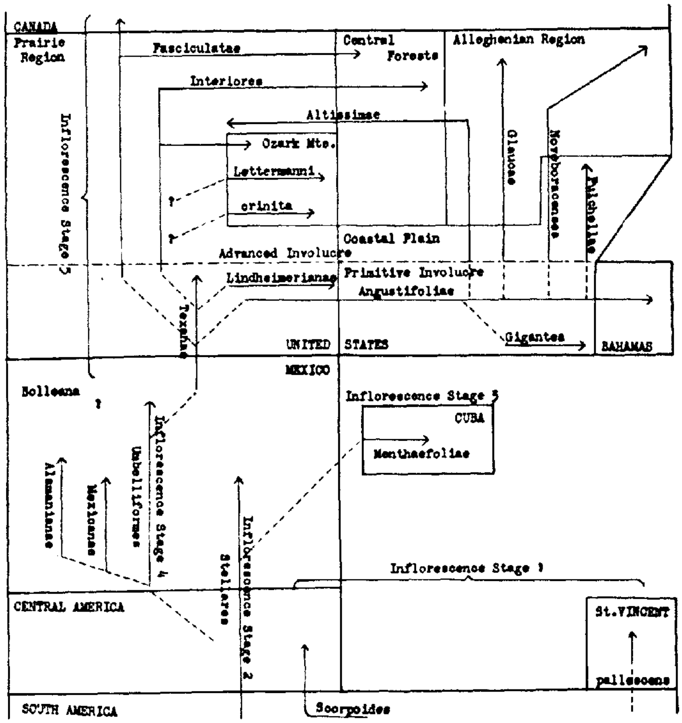


FIG. 3. Migration and evolution of the bractless Vernoniae of North America. Solid lines show distribution by their location, migration by the direction of the arrow. Dotted lines show probable connection between species-group.

occupy outlying ranges or peculiar habitats. The evolution in structure may be summarized as follows:

1. From elongate cymes to short, compact, freely branching cymes or capitate clusters.

2. From small, acute or acuminate involucral scales to broad, blunt, or veiny scales, or to narrow, prolonged, or filiform scales.

3. From a medium number (13–21) of flowers in each head to a large number (55–89, or even more), or to a reduced number (as low as 5).

4. From lanceolate, acuminate leaves to linear, 1-nerved, or revolute leaves, or to blunt, broad, rigid or coriaceous leaves.

In the leafy-bracted forms, comprising 57 species, there is a general

tendency toward the congestion of the inflorescence by repeated branching or toward its reduction by shortening the cymes. The former is most apparent in the species-group Arborescentes, the latter in the Racemosae and Acuminatae. There is a great reduction in the number of flowers in the outlying members of the Acuminatae, Sagraeanae, and Racemosae. Specialization of the involucre by the development of broad, blunt scales occurs in the Schiedeanae, and of narrow, prolonged scales in the Sagraeanae, while the Buxifoliae have increased the number and the regularity of imbrication of the scales. Leaves have shown a tendency to become broad and blunt from the Arborescentes through the Longifoliae and into the Bahamenses, or narrow, one-nerved, and revolute from the Arborescentes into the Racemosae. Montane species have been developed in the Acuminatae and the Buxifoliae, and in both cases are characterized by crowded, few-flowered heads and by small and broad leaves.

Among the 63 species with bractless cymes there is less diversification in structure, except in the inflorescence, which has already been discussed. In the Stellares there is a gradual progression from narrow, acuminate involucral scales to short and blunt ones. A similar tendency occurs among the Interiores and Altissimae, and reaches a climax in the series from the Umbelliformes to the Alamanianae and Mexicanae, with their highly specialized, broad or veiny scales. On the other hand, there is a notable tendency toward prolongation of the scales in *V. crinita* and in the Glaucae, Pulchellae, and Noveboracenses. Excepting the Stellares, all these groups show likewise a tendency to larger heads, reaching a maximum in the Mexicanae, Alamanianae, and *V. crinita*. Forms with unusually small heads rarely occur, and are most characteristic of the single species *V. gigantea*. Two groups only have developed montane forms, the Alamanianae and Mexicanae, and in their unusually large, many-flowered heads differ remarkably from the montane forms of the West Indies.

Neither is there any correlation between structure and habitat. The variation between the montane species of the West Indies and Mexico has already been mentioned. The relatively arid conditions of the Bahamas are reflected in the thick, firm leaves of the Bahamenses and *V. insularis*, but those of the former, with broad-leaved ancestors, are broad and blunt, while the latter, originating from the Angustifoliae of the Gulf States, preserves the linear leaves. The Racemosae, of arid situations in Cuba and Hispaniola, and *V. texana*, likewise a xerophyte, have narrow leaves, but the hydrophytic *V. fasciculata* has narrow leaves also, while the xerophytic *V. Baldwini* has broad leaves. The xerophytic Bahamenses have assumed the form of bushy shrubs, while *V. texana* has remained an herb, although growing in a region where the shrub form is common.

Three processes seem to have been concerned in the general history of the genus, by which it has reached its present distribution and differentiation. Physiological evolution, scarcely indicated by structure, has enabled the

genus to migrate into new environments beyond its original home; migration has brought it to its present distribution and is doubtless still continuing: structural evolution, favored by geographic isolation, has differentiated the present species, but is very little correlated with their physiology or ecology, although proceeding simultaneously with their migration. A single structural tendency appears to be general and possibly orthogenetic, that of the shortening and branching of the cymes.

## Summary

1. Three sections of the genus are represented in North America by a single species each; one section is represented by 120 species.

2. In this section the chief differentiation of groups rests on the structure of the inflorescence; minor differentiation of species-groups is based on the achenes, the involucre, the pappus, and the character of the pubescence.

3. Of the two subsections, one is chiefly Antillean, the other continental, while both are developed in continental South America.

4. Characters which are held to represent primitive conditions in one group may indicate advanced evolution in another, and such characters have no apparent correlation with environment.

5. In every case, those groups which appear to be the simplest in morphological structure occur to the south, while the more complex groups appear progressively farther to the north. In most groups the same statement holds for the individual species.

6. The geographical arrangement of the species-groups and species follows well-known migration routes and supports the conclusion that evolution and migration have proceeded together.

New York Botanical Garden

# THE AVAILABILITY OF IRON IN NUTRIENT SOLUTIONS FOR WHEAT[1]

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The efficiency of the usual trace of iron when employed in culture solutions may be expected to vary with the nature of the compound in which it is supplied, with the composition and reaction of the solution in which it is employed, and with the species of plant. Gile and Carrero have shown that the reaction of the nutrient solution (whether acid, neutral, or alkaline) (6), as well as soil conditions (7), have a marked influence upon the availability of iron to the rice plant. Corsan and Bakke (4) count ferrous iron less efficient than ferric iron, when used in the forms of phosphates. Jones and Shive (9) have pointed out that, in the nutrient solution which they employed, iron in the form of ferrous sulphate was very readily available to the wheat plant, but was evidently somewhat toxic in the highest concentrations used. Ferric phosphate, on the contrary, was very slowly and difficultly available to these plants, even when supplied in relatively large quantities. In later work (10) they found that ferrous sulphate was superior to ferric phosphate as a source of iron for plants grown in a nutrient solution containing calcium nitrate. When ammonium sulphate was employed as the source of nitrogen, however, the solution increased in acidity and ferric phosphate supported growth of the plants better than did ferrous sulphate. Hoagland (8) has suggested that the presence of sufficient dissolved iron in the culture solution will depend upon the form and quantity of the iron salt used, and considers iron citrate and tartrate the most effective forms. Duggar (5) has recommended a special form of "soluble" iron prepared from ferric citrate and sodium phosphate.

## Experimentation

### Solubility

In the present investigation an attempt was made to determine which of the following salts of iron, namely: ferric citrate, $FePO_4$, $Fe_2(SO_4)_3$, and $FeSO_4$, would remain in solution in the greatest amounts at the two hydrogen-ion concentrations of pH 4.2 and pH 6.0, using the Livingston-Tottingham nutrient solution; and also to determine which of these forms of iron would be most available to the wheat plant when supplied in varying amounts, at the latter pH value of the nutrient solution.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported by a grant from the research funds of the University of Wisconsin.

### *Solubility of Iron Salts*

Duplicate one-liter portions of the nutrient solution were prepared from the special salts provided for collaborators (3). To one portion was added enough M/2 KOH to increase the pH value from 4.2 to 6.0. To each of four aliquot portions of the solution at each pH value were added the equivalent of ten milligrams of iron in the form of one of the following compounds: ferric citrate, $FePO_4$, $Fe_2(SO_4)_3$, and $FeSO_4$. The solutions were allowed to stand for 24 hours at a temperature of about 22°C. They were then filtered and evaporated to a volume of about 100 cc. After oxidizing by boiling with the addition of $HNO_3$, the ferric iron was precipitated from the hot solution with $NH_4OH$. A white, flocculent precipitate was obtained in each case. The precipitate was filtered and washed until free from nitrate.

As the amount of iron present in each case was very small, an attempt was made to use the colorimetric method of determining the iron as sulfocyanate (1). Probably because of interference by the salts of the nutrient solution, the results were unsatisfactory.

The following procedure was therefore employed: After dissolving the precipitate in dilute $H_2SO_4$, the iron was reduced by the use of metallic tin and finally titrated with potassium permanganate. The results obtained by this method were fairly satisfactory. The agreement of duplicate solutions may be ascertained from the data given in table 1.

TABLE 1.

TABLE 1. *Solubility of Iron in Livingston-Tottingham Solution $R_5C_1$ when Amounts of Salt Equivalent to 10 Milligrams of Iron per Liter were Employed*

| pH | Ferric Citrate | $FePO_4$ | $Fe_2(SO_4)_3$ | $FeSO_4$ |
|---|---|---|---|---|
| | Mg. Fe per l. | Mg. Fe per l. | Mg. Fe per l. | Mg. Fe per l. |
| 4.2 | 5.7 | 2.3 | 5.6 | 8.6 |
| 4.2 | 6.6 | 2.3 | 5.6 | 9.2 |
| 6.0 | 5.7 | 2.3 | 4.7 | 1.7 |
| 6.0 | 6.3 | 1.7 | 4.5 | 1.4 |

The outstanding feature of these data is the fact that ferric citrate is as soluble in the nearly neutral solution as in the more acid one. $FeSO_4$ is actually the most soluble form of iron at pH 4.2, but its solubility is depressed greatly when the pH value is increased to 6.0. $Fe_2(SO_4)_3$ compares favorably with ferric citrate at pH 4.2, and is not greatly depressed in solubility at pH 6.0. $FePO_4$ is relatively insoluble at both pH values.

### Availability of Iron Salts in Solution Cultures

A series of cultures were arranged to compare the availability of organic and inorganic sources of iron, namely, ferric citrate and $Fe_2(SO_4)_3$; and also

to compare the ferrous with the ferric form of iron, namely, $FeSO_4$ and $Fe_2(SO_4)_3$.

The culture solution used in this experiment was Livingston and Tottingham's $R_8C_1$. The iron was supplied in three different planes as either ferric citrate or $Fe_2(SO_4)_3$, at rates equivalent to 2, 10, and 50 milligrams of iron per liter of nutrient solution. A comparison of $FeSO_4$ and $Fe_2(SO_4)_3$ was made only at the plane of application of 10 milligrams of iron per liter. Six replicative cultures containing five seedlings each were conducted for each plane of iron. A pure strain of Marquis wheat furnished by the Department of Agronomy of the University of Wisconsin was employed.

The method of germinating was as follows: The seeds were immersed in a solution of 0.1 percent mercuric chloride for fifteen minutes, and washed thoroughly with distilled water. They were then soaked in distilled water for 5 to 6 hours, after which they were spread upon mosquito netting which had been paraffined and stretched over granite-ware pans. The latter were 28 cm. in diameter and 10 cm. deep. They were placed in a bath supplied with tap water for maintaining a temperature of about 25°C. Nutrient solution was supplied continuously from a common reservoir at the rate of about 24 liters per pan per 24 hours. The medium employed was Livingston and Tottingham's solution $R_5C_1$, diluted to 1/10 its usual concentration and adjusted to a pH of 7.5 with M/2 KOH. When the

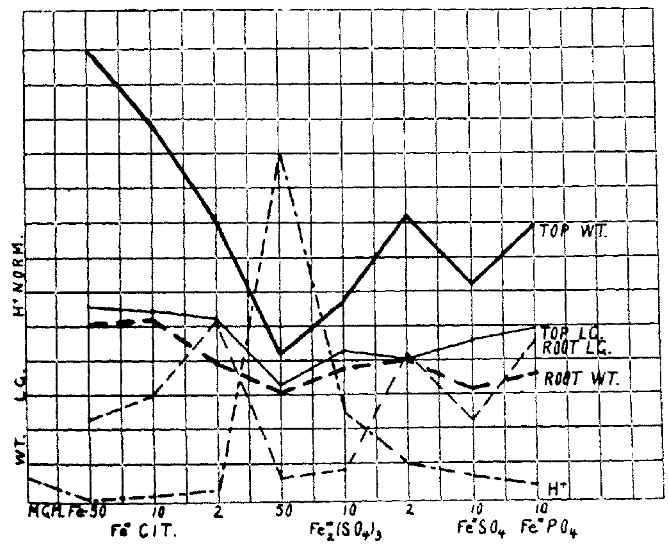


FIG. 1. Relations between sources of iron, acidity of neutral solution, and growth of wheat.

seedlings were about 10 cm. high, specimens of uniform height were selected for use. These were mounted in wide-mouthed jars of about 960-cc. capacity (quart Mason jars), covered with heavy paper so as to exclude light and restrict the growth of algae. The cultures were supported on rotating tables for the purpose of equalizing the exposure of all to variations of light and temperature. This procedure is explained in the manual for collaborators (3).

The experiment continued from April 14 to May 2, 1922. No records were taken of climatic conditions within the greenhouse. However, the thermostat of the house was adjusted for 15.5° C. night temperature. The conditions of solar radiation by day may be considered from the following data derived from records of the local weather bureau station:

Daily percentage of sunshine:
Max. 100.0, Min. 0.0, Mean 58.3.
Daily calories per sq. cm. per hour:
Max. 674.0, Min. 49.0, Mean 472.6.

The pH values of the solutions with various amounts of iron added were determined in the manner described by Clark (2). These values appear in table 2.

TABLE 2. *Values of Solution $R_5C_1$, pH Value 4.2, with Various Forms and Amounts of Iron added*

| | Ferric Citrate | | | $Fe_2(SO_4)_3$ | | | $FeSO_4$ | $FePO_4$ |
|---|---|---|---|---|---|---|---|---|
| Mg. Fe per l. | 50 | 10 | 2 | 50 | 10 | 2 | 10 | 10 |
| pH | 5.0 | 4.8 | 4.4 | 3.0 | 3.6 | 4.0 | 4.2 | 4.4 |

From these data it appears that $Fe_2(SO_4)_3$ increased the hydrogen-ion concentration of the nutrient solution while ferric citrate decreased it. The pH value of certain solutions was determined also after the growth of plants over the 3-day period ending April 28. It was found that, when the two higher planes of $Fe_2(SO_4)_3$ were added, the pH value increased from 3.0 to 3.2 and from 3.6 to 4.0 for the 50-milligram and 10-milligram planes respectively. In all other cases the change of pH value was inappreciable. The plants were so small, however, that they could hardly be expected to exercise much effect upon the composition of the nutrient solution. Certainly, as regards form and appearance of the roots, the increased acidity from the use of $Fe_2(SO_4)_3$ was injurious to the plants.

Photographs of selected cultures were taken on May 1. These are reproduced in figures 2–4. On May 2, after 18 days of growth, the cultures were harvested and the usual separation of tops from roots was accomplished. The data of growth measurements are given in table 3.

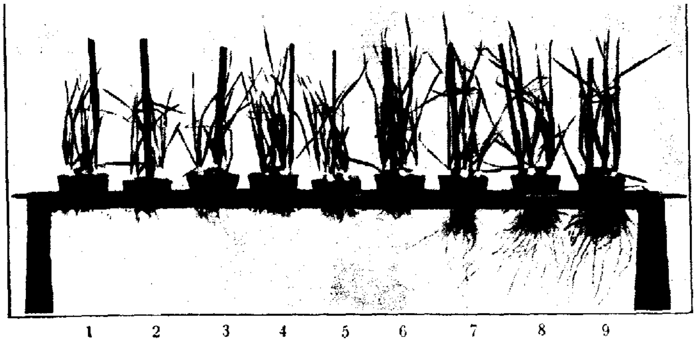


FIG. 2. Cultures 1, 2, 3, $Fe_2(SO_4)_3$, 50 mg. Fe per liter. Cultures 4, 5, 6, $Fe_2(SO_4)_3$, 10 mg. Fe per liter. Cultures 7, 8, 9, $Fe_2(SO_4)_3$, 2 mg. Fe per liter.


FIG. 3. Cultures 1, 2, ferric citrate, 10 mg. Fe per liter. Cultures 3, 4, $Fe_2(SO_4)_3$, 10 mg. Fe per liter. Cultures 5, 6, $FeSO_4$, 10 mg. Fe per liter. Cultures 7, 8, $FePO_4$, 10 mg. Fe per liter.

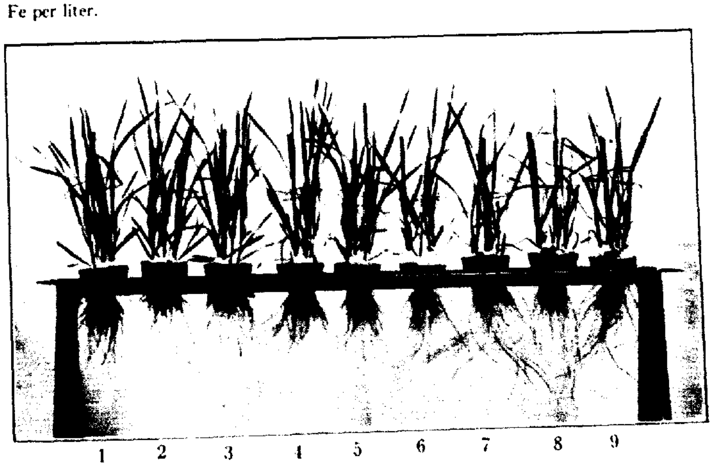

TABLE 3. *Data of Growth of Wheat in Solution $R_3C_1$, with various Planes of Added Iron*

| Source of Iron | Length of Tops in mm. | Length of Roots in mm. | Weight of dry Tops in mg. | Weight of dry Roots in mg. | Comments |
|---|---|---|---|---|---|
| Ferric citrate, 50 mg. Fe per l. ...... | $223 \pm 6$* | $92 \pm 25$ | $517 \pm 43$ | $280 \pm 57$ | Tops dark green, roots yellowish and thick. |
| Ferric citrate, 10 mg. Fe per l. ...... | $221 \pm 31$ | $121 \pm 13$ | $433 \pm 85$ | $206 \pm 21$ | Tops dark green, roots white. |
| Ferric citrate 2 mg. Fe per l. ...... | $209 \pm 13$ | $206 \pm 25$ | $321 \pm 50$ | $157 \pm 26$ | Tops dark green, older leaves dead, roots white. |
| $Fe_2(SO_4)_3$, 50 mg. Fe per l. .......... | $133 \pm 13$ | $25 \pm 10$ | $168 \pm 32$ | $123 \pm 56$ | Tops yellowish green between fibro-vascular bundles, many older leaves dead, roots thick and blackened. |
| $Fe_2(SO_4)_3$, 10 mg. Fe per l. .......... | $171 \pm 13$ | $31 \pm 6$ | $229 \pm 70$ | $153 \pm 28$ | Tops light green, roots yellowish. |
| $Fe_2(SO_4)_3$, 2 mg. Fe per l. .......... | $161 \pm 13$ | $167 \pm 13$ | $328 \pm 63$ | $162 \pm 28$ | Tops dark green but paler than those of citrate-iron cultures, roots white, numerous laterals evident. |
| $FeSO_4$, 10 mg. Fe per l. .......... | $184 \pm 12$ | $90 \pm 6$ | $250 \pm 31$ | $125 \pm 5$ | Tops pale green, roots white, numerous laterals evident. |
| $FePO_4$, 10 mg. Fe per l. .......... | $195 \pm 12$ | $183 \pm 13$ | $315 \pm 70$ | $144 \pm 23$ | Tops dark green, older leaves dead, roots white. |

* Average departure from the mean of six cultures.

## Discussion

The results of the solubility test here reported show decided differences between the various forms of iron employed. It was found that $FePO_4$, a form commonly employed in nutrient solutions, was relatively insoluble at both pH values tested. $FeSO_4$, while the most soluble of the forms compared at the higher hydrogen-ion concentration, was relatively insoluble at the lower one. This result at the lower hydrogen-ion concentration would seem to indicate hydrolysis with the formation of insoluble $Fe(OH)_3$ or basic ferric salt. McCall and Haag (12) have called attention to this factor in the availability of iron in nutrient solutions. While $Fe_2(SO_4)_3$ compares favorably with ferric citrate in solubility, it increased the hydrogen-ion concentration of the nutrient solution to what seems to have been an unfavorable extent. It was also slightly depressed in solubility in the less acid solution. Ferric citrate appears to possess a fair degree of solubility over a considerable range of pH value of the nutrient solution. With the

hydrogen-ion concentration of the iron-free nutrient solution at pH 4.2, the growth of young wheat plants shows ferric citrate to be decidedly the most available form of iron. In this form, 10 milligrams of iron per liter appear to be sufficient for the growth of these plants.

With the exception of the less favorable results from $FeSO_4$ as compared with its high solubility, the variations of efficiency in these various forms of iron can be attributed either to differences in solubility or to different modifying effects upon the pH value of the nutrient solution. Thus, ferric citrate reduces the hydrogen-ion concentration and acts favorably, while $Fe_2(SO_4)_3$ increases the concentration of this ion and is an injurious source of iron. The inferiority of $FeSO_4$ as compared with ferric citrate may reside in toxic properties of the former, as suggested by Jones and Shive (9).

It seems probable that the formation of either $Fe(OH)_3$ or basic iron salts by hydrolysis of inorganic salts of iron will render these unavailable in general as sources of iron in nutrient solutions. Yet ferric hydroxide may be superior to ferric phosphate as a source of iron in some cases, as indicated by a previous observation (13) that barley seemed to acquire a much improved supply of iron from a nutrient solution containing suspended ferric phosphate when ferric hydroxide was also added. Apparently the use of $FePO_4$ should be discriminative, because of its low solubility. These results confirm previous observations of the favorable effects of iron applied to nutrient solutions in the form of salts of organic acids. The conclusions find support in both the appearance of plants, especially as to intensity of green color of the leaf, and the data of plant measurements.

## Summary

1. Results are here given relative to the solubility and availability to young wheat plants of various compounds of iron in a particular form of nutrient solution.

2. Solubility tests at different pH values of the nutrient solution have shown that ferric phosphate is relatively insoluble. This is true of ferric and ferrous sulphate at a hydrogen-ion concentration of the iron-free nutrient solution approaching neutrality. While ferric citrate is not very soluble, it possesses the advantage of remaining soluble over a considerable range of pH values of the nutrient solution.

3. Ferric sulphate increases the hydrogen-ion concentration of the nutrient solution here used, while ferric citrate causes the opposite effect. The other forms of iron tested have little influence in this respect.

4. The growth measurements of the young wheat plants show that ferric citrate was decidedly the most favorable form of iron here employed. The variation in efficiency of iron in the forms supplied is correlated with variation either in the solubility of this element or in the modification of the pH value of the nutrient solution. The results show clearly that ferric phosphate is likely to be inefficient because of its low solubility.

5. Ferric citrate supplied at the rate of 10 milligrams of iron per liter of the nutrient solution employed here is not completely dissolved, but seems to provide abundant iron for the growth of the young wheat plant, where the nutrient solution is renewed.

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# THE EFFECT OF THE HYDROGEN ION ON THE PROTOPLASM OF THE ROOT HAIRS OF WHEAT

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In modern experiments with nutrient solutions in which phosphates are provided as either potassium mono-hydrogen or potassium di-hydrogen phosphate, it has been found by several writers that in fairly high concentrations these phosphates are injurious. Hoagland ('17), Duggar ('20), and Salter and McIlvaine ('20), indeed, found that if the concentration of phosphate was increased to but a slight extent, its injurious effect was marked. Since phosphorus is essential for the growth of plants, and is actually a constituent of protoplasm, it is not at once clear why its influence should so suddenly change from beneficial to harmful as its concentration is increased in the culture solution. The unquestioned nutritive value of the phosphate ion and of the potassium ion directs attention to the other component of the salt, the hydrogen ion. A logical first step in determining the nature of the harmful effect might be to ascertain by inspection whether it is general or local; to see if any particular cells behave or develop differently in solutions containing a large amount of phosphate than in solutions in which the concentration is lower.

For this purpose seedlings of spring wheat (*Triticum vulgare*), "Marquis" strain, were grown in solutions selected from Shive's ('15) "optimal" group. The total osmotic pressure of each of these solutions was calculated by Shive to be about 1.75 atmospheres. Since the lime-magnesium ratio has been thought to influence the growth of plants,[1] solutions were chosen in which this ratio was practically constant. The compositions and hydrogen-ion concentrations of the solutions employed are shown in table 1.

The hydrogen-ion concentrations were determined colorimetrically with a colorimeter of the form described by Bock and Benedict ('18). The indicator used in these experiments was tetrabromphenolsulphonephthalein (brom-phenol blue), prepared according to the directions of Clark ('21). The buffer solutions used were mixtures of potassium hydrogen phthalate and hydrochloric acid, and of potassium hydrogen phthalate and sodium hydroxide (Clark, '21); they were standardized by titration with dibromthymolsulphonephthalein (brom-thymol blue) against standardized hydrochloric acid obtained from the LaMotte Chemical Products Company of Baltimore, Maryland.

Baker's "analyzed" chemicals were used for the culture solutions, which were made up from single-salt stock solutions drawn from covered

[1] For a general discussion see Lipman ('16).

burettes and diluted to the required volume with water that had been condensed in block tin and stored in a paraffin-lined jar.

TABLE 1. *Composition and Hydrogen-ion Concentration of Solutions Employed*

| Solution Number | Shive's Designation | Hydrogen-ion Concentration pH[2] | Composition Partial Concentration in Grams per Liter of Solution | | |
|---|---|---|---|---|---|
| | | | $KH_2PO_4$ | $CaNO_3.4H_2O$ | $MgSO_4.7H_2O$ |
| 1.... | $R_2C_4$ | 3.94 | 0.98028 | 2.45600 | 4.92984 |
| 2..... | $R_4C_3$ | 3.85 | 1.96056 | 1.84200 | 3.69738 |
| 3..... | $R_5C_2$ | 3.68 | 2.45070 | 1.22800 | 3.69738 |
| 4..... | $R_6C_2$ | 3.60 | 2.94084 | 1.22800 | 2.46492 |
| 5..... | $R_8C_1$ | 3.47 | 3.92112 | 0.61400 | 1.23246 |

3 drops of a 0.001 M solution of $Fe_2Cl_6$ were added to every 325 cc. of nutrient solution.

The seeds were germinated on moist, unglazed pottery, and were then placed on paraffined cotton netting stretched over the tops of glass tumblers of tap water, where they remained for three or four days, or until the seedlings were from four to six centimeters high. From these, plants were selected for uniformity in size, color, and development, and were transferred to culture bottles containing the nutrient solutions, which were changed at intervals of from three to five days. The culture bottles were of flint glass, with wide mouths and cork stoppers, and had a capacity of about 325 cc. Each was enclosed by two jackets, the inner one of heavy black paper, the outer one of heavy light-colored manila paper. Four holes were bored in each stopper, which was then immersed for a few minutes in hot paraffin, and one plant was fixed in each hole with a pledget of non-absorbent cotton. The cultures were kept in a greenhouse at a temperature of 20° to 25° C. throughout the winter and spring months.

Although differences in the length and general appearance of the plants were not noticeable during the first few days that the plants grew in the nutrient solutions, there were appreciable differences in the root systems before the third day. The roots of plants in solution 1 were long and straight with several long secondary roots; those in solutions 4 and 5 were short and stubby. Some of the roots in solution 5 were branched very near the tips; these branches were hardly more than tubercles. The secondary roots in solutions 4 and 5 were thick, much branched, and bore an unusually large number of root hairs, some of which were so long that their ends projected beyond the tip of the root itself. Plants in solutions 2 and 3 presented appearances intermediate between the two extremes; the roots were of medium length and were moderately branched, but they were not stubby. At the end of a week the differences between cultures were even more apparent. It thus appears that short, stubby, branched root systems are associ-

[2] These pH values are somewhat smaller than those obtained by McCall and Haag ('20) and by Meier and Halstead ('21). However, since it is the relative concentrations that are of value in the present experiments, the actual pH is unimportant.

ated with high concentrations of potassium di-hydrogen phosphate and consequent high hydrogen-ion concentration. Excessive branching of roots and stimulation of the growth of root hairs have been observed by Hoagland ('17), although he does not mention the characteristic stubbiness.

This injury to the roots, which antedates injury to the tops, conceivably and apparently renders the roots unable adequately to perform their functions, and presumably thus causes the later decreased growth of tops observed by other writers. Since the substances in the nutrient solution, as they pass into the plant, encounter the root hairs first, the effect upon the protoplasm of these cells was investigated, and the problem was accordingly narrowed at this point in the investigation to the changes that take place in root hairs.

Protoplasm consists, at least chiefly, of substances in the colloidal state. This has been known ever since the dark-field microscope came into use among biologists, although the precise nature of the colloids is as yet undetermined. Gaidukov ('10) described protoplasm as a sol because he observed many characteristics—for example, Brownian movement—that suggested a liquid condition. Since this pioneer work was published, however, studies of a greater variety of cells, and of the same cell under different external conditions, have shown that protoplasm is not to be so briefly characterized. Seifriz ('20) disagrees with Kite ('13) in his belief that protoplasm is ever so rigid that it can be cut into pieces that do not change in shape, and states that its cohesion is never greater than that of a plastic and viscous jelly. He found by micro-dissection that the streaming protoplasm of Rhizopus is fluid, while the endoplasm of Amoeba is decidedly viscous. After experimenting with protoplasm from many kinds of cells, both plant and animal, he concludes that within a given cell the viscosity decreases as the protoplasm becomes more active, and increases as it becomes more quiescent. This phenomenon had already been observed by Price ('14), who noted that the protoplasm of Mucor spores changes from jelly to sol as the spores germinate, and that the protoplasm of Fucus eggs changes from sol to jelly as the eggs mature. The general conclusion to which the work of these and other investigators points is that the viscosity of protoplasm is not constant; it is different in different cells, it may be different in the same cell at different times, and, indeed, it is highly probable that it is different in different parts of the same cell at the same time.

Chemically, protoplasm probably consists of a "complex of substances of various chemical natures and in various states of aggregation, associated by forces of surface tension, electrical charge, and so forth."[3] The exact nature of this complex is as yet undetermined: Lepeschkin ('13) believes it to consist largely of proteins and lipoids, while MacDougal and Spoehr's ('20) "bio-colloids," which simulate in many ways the colloidal behavior of protoplasm, are mixtures of proteins and pentosans, and are greatly in-

[3] Bayliss ('18), p. 26.

fluenced by amino acids. It may be that there are two essentially different substances in protoplasm, one more viscous than the other, and that various substances are distributed in these two media as non-living inclusions. It is also possible that living protoplasm exists in only small quantities in any cell, while the greater share of the substance now termed protoplasm is composed of products of the cell enzyms; products which are themselves non-living, but which, because they may change their colloidal state readily, may often appear as the conspicuous part of the entire system. Coagulation and variations in viscosity observed in "protoplasm" might thus be due to colloidal changes in non-living inclusions. Whatever value these suggestions may have, the data are at present insufficient to decide between them. Irrespective of just what it is that coagulates, coagulation is a sign that the cell is becoming more inactive. For the descriptive purposes of this paper, "protoplasm" will be considered to mean the entire visible colloidal complex. It varies in viscosity from a sol to a stiff jelly, and it contains or is associated with proteins, carbohydrates, fats, lipoids, and salts, in unknown combinations. Because it can form jellies, and because more than a trace of electrolyte is required to precipitate it, it is classified with the emulsoids.

The part that is played by the surface layer of a protoplast in determining the permeability of protoplasm is not known. By some writers it has been regarded as a membrane of peculiar composition; by others as merely a condensation film of protoplasm, differing from the interior of the protoplast in its surface-tension qualities. Of course the latter hypothesis cannot hold, for the Gibbs-Freundlich law, which states that substances that lower the surface tension of a system become more concentrated at the surface, and that substances that raise the surface tension become more concentrated in the interior, applies to solutions only. Protoplasm is obviously not a solution, and we have no knowledge of the laws that govern the distribution of substances in a heterogeneous, colloidal system. Whatever the nature of the surface layer may be, it is to be remembered that it is possible for a substance in solution to affect the structure of a protoplast whether it enters it or does not enter it. If it enters, its effect will be determined by the nature and amount of the substance, the condition of the protoplasm, and the natures and relative amounts of the substances in the protoplasmic layer and in the cell sap. It might penetrate the protoplast without changing it; it might alter the colloidal structure by reacting with the constituents of the protoplasm or merely of its surface layer; or it might change the electric signs of the colloids. If the substance does not enter the protoplast, it might react with substances in the surface layer, or by adsorbing ions it might change the electric signs of the colloids, and thus affect the colloidal structure without having entered the protoplast. It is apparent, then, that the terms "permeability" and "impermeability" are applied rather loosely to the perceptual results of several different processes. Physiologists have not yet been able to determine for given

situations just what processes actually operate. Indeed, unless a substance is so colored that its presence can be actually seen in the protoplast, or unless its passage can be traced through the plant, the experimenter does not even know whether or not the substance entered the protoplast of which the structure is affected. In order to determine what process operates in a given situation, he must know just how the given substance affects the given protoplast. Very little of this kind of work has been done.

The dark-field microscope offers the best method for this kind of study, because it makes possible observation of colloidal particles which are too small to be resolved by the ordinary microscope. The apparatus used in the present investigation consisted of a compound microscope fitted with a Zeiss cardioid condenser, a 1.8-mm. achromatic objective, and a 10 x ocular. The cardioid condenser requires intense illumination because the cone of light becomes very broad before entering the objective. A small arc lamp with carbons fixed at an angle of 70° was found satisfactory. The light was passed through an aqueous ammoniacal solution of copper sulphate to remove the red rays, and was then focused on a plane mirror, from which it was reflected into the condenser. The slides and cover glasses were selected for a uniform thickness of 1.0 and 0.1 mm. respectively. They were cleaned with alcohol, dipped in collodion, dried, and then stored in water; immediately before a slide or a cover glass was used, it was wiped free from water and the thin film of collodion was stripped from the surface, thus removing all dust particles.

The protoplasm of an actively growing root hair appears milky under the dark-field microscope because the particles are so small, so numerous, and so evenly distributed that the enlarging light cones overlap, thus making it impossible to distinguish the individual particles. In a very young root hair the protoplasm is dense and almost devoid of vacuoles. As the root hair grows, the protoplasm becomes less dense, vacuoles form and enlarge, and the cell is apparently at the height of its usefulness as an absorbing organ. The vacuoles continue to enlarge and begin to coalesce, and the protoplasm is crowded more and more toward the outside of the cell, so that finally it is but a thin film separating the cell sap from the cell wall, and the root hair is of little value to the plant. To study the effects of nutrient solutions on the protoplasm of root hairs, it is thus obviously necessary to select cells that are in the second stage of their grand period of growth, for at this age their condition determines their value to the plant.

Root hairs of wheat grown in the nutrient solutions described above differ markedly in the appearance of their protoplasm, when they are examined at the age of approximately maximum usefulness to the plant. These differences are shown in figure 1, Plate XXIII. In hairs from plants grown in solution 1 (pH 3.94) the protoplasm is evenly distributed through the cell and shows no suggestion of precipitation[4] or of aggregation into masses.

[4] To avoid ambiguity, it becomes necessary to define the following terms, which have been used in different senses by different colloid chemists:

Hairs from plants grown in solutions 2 and 3, of which the hydrogen-ion concentration is higher (pH 3.85 and 3.68 respectively), contain more vacuoles, but there is no indication of coagulation. Decided differences are apparent between the protoplasm of root hairs grown in solutions 4 and 5, which are still more acid (pH 3.60 and 3.47 respectively), and that of the root hairs mentioned above. Hairs from solution 4 show the beginning of gel-formation: a coagulum has begun to form, the dispersion has decreased sufficiently to enable one to distinguish individual particles, and these are becoming flocculated into larger masses; however, there are still large portions of the protoplast that do not show coagulation. In hairs from solution 5, gel-formation is more pronounced: coagulation and flocculation are evident throughout the protoplast. In some places the flocculent masses are collected in the interior of the cell, thus indicating that the root hair is not turgid and is consequently of no value as an absorbing organ. Some protoplasm is still unprecipitated, but the amount is so small that it is almost imperceptible because of the contrast between the dark background and the white, flocculent masses of irreversible gel.

As has been noted above, this gel-formation occurs in the root hairs taken from cultures that have a large phosphate content, and consequently a large hydrogen-ion content, and it does not occur in root hairs grown in solutions containing a smaller proportion of the phosphate. Since the calcium-magnesium ratio is the same in all these solutions (except solution 3, which, although it differs slightly in calcium-magnesium ratio, was included in the present experiments because it is generally considered Shive's "best" solution), and since there is no evidence to show toxicity on the part of any of the other ions in these solutions, the conclusion that the gel-formation is due to the hydrogen ion seems inescapable.

This correlation is further supported by experiments that were performed to determine the effect of acids on protoplasm. In these experiments the hydrogen-ion concentration was varied, not by changing the salt proportions in nutrient solutions, but by irrigating the root hairs with solutions of an inorganic acid in pure water. The seeds were germinated on porous pottery kept moist with tap water, and the plants were taken directly from the pottery germinators for study when they were two or three days old. In this drier habitat they produced an abundance of root hairs. Moreover, the protoplasm in these root hairs was apparently denser, for under the dark-field microscope they appeared whiter. The osmotic pressure of the root hairs was also increased, for they were not plasmolyzed by a 0.45 M solution of hydrochloric acid, sodium chloride, or nitric acid.

A *gel* is a solid formed from a sol or a jelly by the action of heat or of chemical reagents, *i.e.*, by processes other than mere loss of water. It is irreversible because the sol or jelly state cannot be regained by addition of water.

*Precipitation* or *coagulation* is the formation of a gel. It may be accompanied with a decrease or an increase in the dispersion of the particles.

*Flocculation* is the aggregation of precipitated particles into large, soft masses that remain suspended in the medium.

The roots, with root hairs, were mounted under the dark-field microscope in aqueous solutions of acid, and were irrigated with the same solution from time to time. Figure 2 shows successive stages in the precipitation of the protoplasm of one of these hairs that was subjected to the action of a 0.45 M solution of hydrochloric acid during a period of five hours. The similarities in appearance of this hair and of those previously described should be noted, especially since in this hair successive stages in the reaction between the hydrogen ions in relatively high concentration and the protoplasm of a single hair is being followed, whereas the drawings in figure 1 show different root hairs that were acted upon by hydrogen ions in different concentrations. Coagulation appears first at the vacuolar membranes and spreads through the protoplasm, enlarging the vacuoles as flocculation proceeds. The process is identical with that in the root hairs that were grown in nutrient solutions containing a large amount of phosphate, except that the flocculi are larger and the dispersion medium is clearer when the precipitation is affected by the hydrochloric-acid solution. Quantitative differences are to be expected, for the hydrogen-ion concentration is higher.[5] Moreover, in the nutrient solutions two processes may be considered to be operating—one induced by the nutrient ions, tending to maintain the normal structure of the protoplasm, and the other induced by the hydrogen ions, tending to coagulate it. In the hydrochloric-acid solution there are practically no nutrient ions, so that the coagulation by the hydrogen ions is not repaired. This so-called "antagonism" of ions is well known, although the processes affected are not always the same.

In order to determine whether or not coagulation was due to a specific effect of the hydrogen ion, the same experiment was performed with a 0.45 M solution of sodium chloride instead of hydrochloric acid. The results are shown in figure 3. At the end of five hours the protoplasm showed no change except that it had become slightly more vacuolated. Since the two equivalent solutions differ as to cation but not as to anion, it seems clear that the coagulation caused by the hydrochloric acid was induced by the hydrogen ion. This inference has led some investigators [McCall and Haag ('20), Meier and Halstead ('21)] to seek a direct relation between hydrogen-ion concentration and the yield of plants, but they were unable to find such a relation, although still convinced that the hydrogen ion does exert an influence on plant growth. The nature of this influence forms the subject of this paper, and it will emerge that this influence, although it is decidedly effective, is of such a kind that there can be no direct relation between the hydrogen-ion concentration of nutrient solutions and the yield of plants grown in them.

The conclusion that the coagulation of the protoplasm was induced by the hydrogen ion becomes still more probable when the close similarity in appearance between this cell and those protoplasts that were coagulated

[5] The pH of a 0.45 M solution of HCl is approximately 0.4.

in the culture solutions is noted, for protoplasm is coagulated differently by different reagents. For instance, osmic acid characteristically produces a net-like coagulum,[6] which differs materially from the flocculum observed in the present experiments.

Although the argument above presented seems logical, it may be that the coagulation was induced by the chlorine ion, and that the sodium ion rendered it innocuous when sodium chloride was used instead of hydrochloric acid. The experiment was accordingly repeated with a 0.45 M solution of nitric acid, the anion of which was formed in the nutrient solutions by the dissociation of calcium nitrate. The results, shown in figure 4, duplicate the results that were obtained with hydrochloric acid and with the nutrient solutions of high phosphate concentration. The conclusion seems inescapable that in every instance the coagulation is due to the hydrogen ion.

A further experiment was undertaken to determine whether or not the presence of salts alters the rate of coagulation by the hydrogen ion. Root hairs were irrigated with solution 2 to which sufficient nitric acid had been added to make the concentration of acid 0.45 M, and note was taken of the time required to produce a degree of coagulation equal to that produced by the end of four and one half hours when an equimolecular solution of nitric acid in pure water was used. Of the two root hairs irrigated with nitric acid in salt solution, the protoplasm of one was coagulated to the extent defined above in four hours, that of the other in five hours. Since these results indicate a pronounced individual difference in root hairs, and since the effect of salts on the chemical potential of the hydrogen ion is not properly a part of the present thesis, the experiments were discontinued. The experiment is cited, however, and drawings of one of these hairs are shown (fig. 5), because, since the coagulation was produced in a nutrient solution, and since it is of the same kind as that usually produced in solutions of high hydrogen-ion concentration, although to a greater extent because of the added acid, it affords additional evidence, if that be needed, that the coagulation in the culture solutions is due to the specific effect of the hydrogen ion.

It is now possible to explain the abnormal root development in those nutrient solutions that contain an injurious quantity of potassium di-hydrogen phosphate. The hydrogen ions precipitate the protoplasm of the root hairs, which are the primary absorbing cells of the plant, thus increasing their permeability and rendering them unable to act as absorption organs. As the root hairs become ineffective, more are formed, as in dry soils or in culture solutions such as solutions 4 and 5, where the phosphate concentration is too high. If the root cannot produce enough root hairs to carry on the work of the plant, which in turn becomes stunted, then more roots are formed, thus producing the short, branched root systems described above. This series of effects affords another[7] example of the Le Chatelier-Braun

[6] Bayliss ('18), p. 15.

[7] For other examples, see Bancroft ('11).

theorem [Le Chatelier ('84), Braun ('87)], which states that a system affected by an outside condition tends to alter within itself in such a way as to oppose and partially annul the effects of this outside condition.[5] It thus becomes evident that there can be no direct relation between the hydrogen-ion concentration of nutrient solutions and the yield of plants grown in them, for the hydrogen ion affects the absorbing action of the plant, which in turn affects, not any one plant measurement, but all directions of growth.

## Summary


1. The abnormal root development and decreased growth that have been observed in plants grown in nutrient solutions that contain relatively large amounts of potassium di-hydrogen phosphate may be explained by the coagulation of the protoplasm of the root hairs.

2. This coagulation, which is accompanied with flocculation, is found to be induced by the hydrogen ions formed by dissociation of the phosphate. The hydrogen-ion concentration of the nutrient solutions employed varied from pH 3.94 to pH 3.47.

3. The relation of this coagulation and flocculation to the colloid chemistry of protoplasm is discussed.

4. The lack of logic in the attempts of certain investigators to find a direct relation between environmental features, such as hydrogen-ion concentration, and the dry weight of plants, is pointed out.


In concluding, I wish to express to Professor Howard E. Pulling of Wellesley College sincere gratitude and appreciation for many suggestions, constant interest, and other assistance during the progress of the experiments and the preparation of this paper.

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## EXPLANATION OF PLATE XXIII

The optical apparatus used consisted of cardioid condenser (Zeiss), 1.8 mm. achromatic oil-immersion objective (Spencer), and 10 × ocular (Spencer). In the figures a camera lucida (Bausch and Lomb) was used in drawing the outlines and indicating the general regions of the cell content. Details were drawn free-hand. The magnification of the figures is approximately 810 diameters.

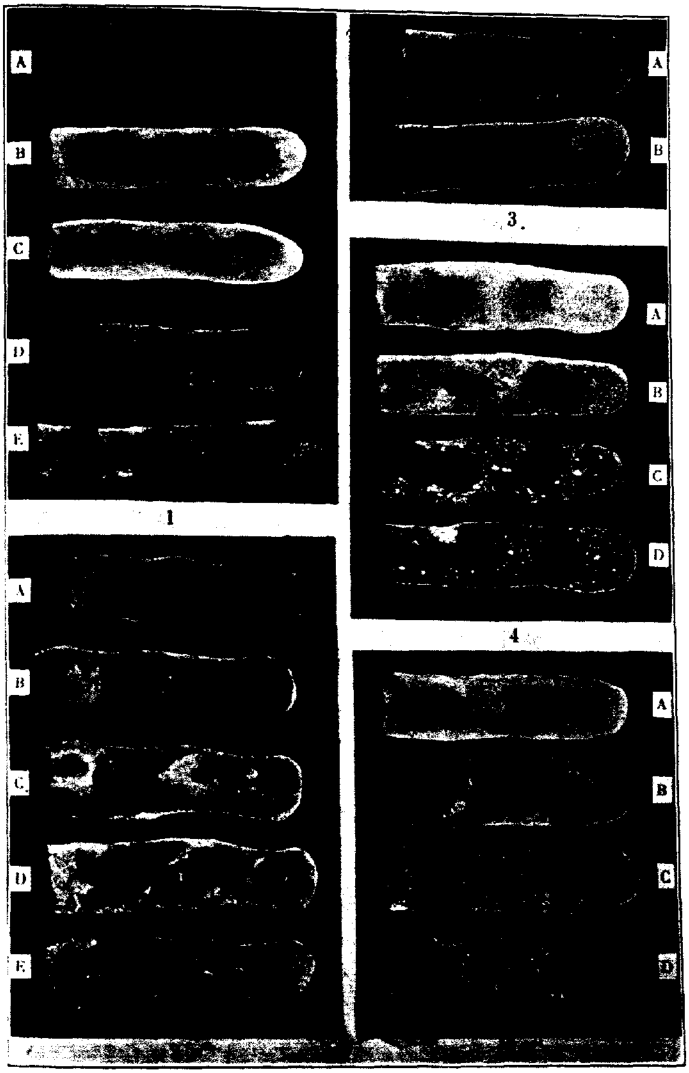
FIG. 1. Root hairs of wheat grown for one week in the following solutions: *A*, solution 1. *B*, solution 2. *C*, solution 3. *D*, solution 4. *E*, solution 5.

FIG. 2. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of hydrochloric acid in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of ½ hour. *C*, at the end of 1½ hours. *D*, at the end of 2½ hours. *E*, at the end of 5 hours.

FIG. 3. Root hair of wheat, showing the effect on the protoplasm of a 0.45 *M* solution of sodium chloride in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of 5 hours.

FIG. 4. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of nitric acid in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of 1½ hours. *C*, at the end of 3½ hours. *D*, at the end of 4½ hours.

FIG. 5. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of nitric acid in culture solution 2. *A*, immediately after being mounted in the solution. *B*, at the end of 1½ hours. *C*, at the end of 4 hours. *D*, at the end of 5 hours.




ADDOMS: HYDROGEN ION AND PROTOPLASM